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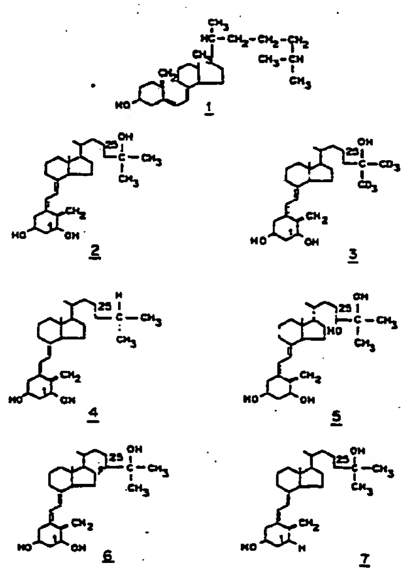

(57) Abstract

Methods and compositions for enhancement of phytate phosphorus utilization and treatment and prevention of tibial dyschondroplasia in animals, particularly poultry, by administering to animals a feed composition containing a hydroxylated vitamin D_3 derivative. The vitamin D_3 derivative is preferably administered to animals in feed containing reduced levels of calcium and phosphorus for enhancement of phytate phosphorus utilization. The vitamin D_3 derivative is administered in combination with active phytase for further enhancement of phytate phosphorus utilization. The figure is a drawing of the chemical structures of vitamin D_3 (cholecalciferol)(1), 1,25-dihydroxycholecalciferol (2), 1,25-dihydroxy-26,27-hexadeuterocholecalciferol (3), 1-hydroxycholecalciferol (4), 1,24,25-trihydroxycholecalciferol (5), 1,25-dihydroxy-24-fluoro-cholecalciferol (6), and 25-hydroxycholecalciferol (7) as used in the methods and compositions described herein.

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VITAMIN D DERIVATIVE FEED COMPOSITIONS AND METHODS OF USE

Background of the Invention

This invention relates to the field of biochemistry, and more particularly relates to animal feed compositions.

Phytate Phosphorus Utilization

Animals, including humans, require phosphorus in their diets for proper growth and health. Farm animals are normally fed a grain-based animal feed. Most of these grain-based feeds contain from 50-80% of their total phosphorus as phytate phosphorus. Phytate phosphorus in plants occurs as the mixed calcium-magnesium-potassium salt of the organic compound, phytic acid.

Many animals are unable to utilize most of the phytate phosphorus they receive in their feed. For example, studies by Edwards and Veltmann, *J. Nutr.* 113:1268-1575 (1983) and Ballam *et al.*, *Poultry Sci.* 63:333-338 (1984) with young broiler chickens fed corn-soybean diets indicate phytate phosphorus utilization of from only 10 to 53%. Feed consumed by these animals must be supplemented with inorganic phosphorus, such as in the form of dicalcium phosphate or defluorinated phosphate. The cost of phosphorus supplementation is high. In addition, the unused phytate phosphorus is excreted, creating phosphorus soil contamination and costly ecological problems.

The mechanisms involved in phytate phosphorus utilization by animals are unknown. Utilization of phytate phosphorus by chickens has been reviewed by several scientists including T.S. Nelson, *Poultry Sci.* 46:862-871 (1967). Phytate phosphorus utilization in broiler chickens has been shown by Edwards *et al.*, *Poultry Sci.* 67:1436-1446 (1988) to be influenced by age. Oth r scientists, such as Lowe and Steenbock, *Biochem J.* 30:1991-1995 (1936), Common, *Agric. Sci.*

30:113-131 (1940), Edwards and V ltmann, *J. Nutr.* 113:1268-1575 (1983), Ballam *et al.*, *Poultry Sci.* 63:333-338 (1984) and Sooncharernying and Edwards, *Poultry Sci.* 69(Suppl. 1):129 (1990), have shown that phytate phosphorus utilization may be influenced by calcium, phosphorus, and aluminum levels in the diet.

A method that would increase phytate phosphorus utilization by animals, especially farm animals such as chickens and turkeys, would reduce the costs of raising such animals because phosphate supplementation would be unnecessary. In addition, the soaring costs associated with the decontamination of soil containing elevated phosphates would be greatly reduced or even eliminated.

Tibial Dyschondroplasia

Tibial dyschondroplasia is a skeletal abnormality which occurs in rapidly growing animals such as broiler chickens or turkeys. The cause of tibial dyschondroplasia is unknown. Tibial dyschondroplasia is distinguished from rickets, a vitamin D deficiency disease characterized by overproduction and deficient calcification of osteoid tissue, in that a high incidence of tibial dyschondroplasia is found in animals that receive a sufficient dietary supply of vitamin D and are adequately exposed to sunlight.

Tibial dyschondroplasia is characterized by an unmineralized, unvascularized mass of cartilage located in the proximal ends of the tibiotarsus and the tarsusmetatarsus. The cartilage extends from the growth plate into the metaphysis. In fowl, tibial dyschondroplasia usually appears between three and eight weeks of age. In some chickens and turkeys, the prehypertrophic cartilage persists into adulthood but is restricted to the posterior medial portion of the proximal tibiotarsal bone so that the birds remain clinically normal. An incidence of 10 to 30% of birds

with subclinical dyschondroplasia is common in many flocks. In the more severe cases of tibial dyschondroplasia, the abnormal tissue occupies the whole metaphysis of the proximal tibiotarsal bone and also develops in the proximal tarsometatarsal bone.

Birds with these more severe lesions may be lame, with bowing of the affected bones. These chickens are unable to walk normally and often collapse, causing injury and decreasing growth rate. The disease also increases the death rate of animals during the growth period. Further, many of the birds suffering from tibial dyschondroplasia develop breast blisters and leg deformities that result in hemorrhages.

Tibial dyschondroplasia increases the percentage of carcasses that must be downgraded or condemned during processing, resulting in decreased profits for the processor. The deformed legs of birds with tibial dyschondroplasia often interfere with the shackling of the fowl during processing and can actually cause mechanical problems in operating the processing line where the slaughtered fowl are conveyed on machines which handle the birds by their legs. Fowl with tibial dyschondroplasia have insufficient leg strength to be carried in this manner.

A number of studies have been conducted to determine both the cause of dyschondroplasia and a method for treatment or prevention. Leach and Nesheim, "Further Studies on Tibial Dyschondroplasia Cartilage Abnormality in Young Chicks", *J. Nutr.* 102:1673 (1972), indicated that the cartilage abnormality is a result of an inherited physiological defect, the expression of which is under dietary control. They were not able to determine the nutritional factors responsible for expression. However, they found that manipulations of the mineral mixture that resulted in changes in acid/base or cation/anion balance in the

chick altered the incidence of abnormality. In particular, high chloride level in the diet increased the incidence of the abnormality.

Mongin and Sauveur, in "Interrelationship Between Mineral Nutrition, Acid-Based Balance, Growth and Cartilage Abnormalities," Growth and Poultry Meat Production, Borman, K. N. and Wilson, B. J., Eds., pp. 235-247, British Poultry Science Ltd., Edinburgh, Scotland (1977), hypothesized that the metabolic acidosis in chickens fed high dietary chloride levels caused tibial dyschondroplasia because of impaired bone mineralization resulting from alteration of vitamin D metabolism.

In most animals, including humans, vitamin D_3 (cholecalciferol) is metabolized first by hydroxylation at the C_{25} position to 25-hydroxycalciferol by one or more enzymes present in the liver, and then hydroxylation of the 25-hydroxycalciferol at the C_1 position to calcitriol (1,25-dihydroxycholecalciferol) by one or more enzymes present in the kidneys. A flow chart showing the chemical structures involved in vitamin D derivation and metabolism is shown in Figure 1.

Chickens made acidotic by administration of ammonium chloride show reduced conversion of $25(OH)D_3$ to $1,25(OH)_2D_3$, although the production of 24,25-dihydroxycholecalciferol ($24,25(OH)_2D_3$) from $25(OH)D_3$ is not consistently affected by acidosis, as also reported by Sauveur and Mongin in "Influence of Dietary Level of Chloride, Sodium and Potassium on Chick Cartilage Abnormalities," *Proceedings of XV World Poultry Congress*, pp. 180-181 (1977).

However, supplementation of chickens with 20 ng/day of either 1,25-dihydroxycholecalciferol ($1,25(OH)_2D_3$) or 24,25-dihydroxycholecalciferol ($24,25(OH)_2D_3$) has been demonstrated to have no effect

on the incidence of tibial dyschondroplasia, as described by Edwards in "Studies on the Etiology of Tibial Dyschondroplasia in Chickens", *J. Nutr.*, 114:1001 (1984).

Calcium and phosphorus levels in the diet have been found to be major nutritional factors influencing the expression of tibial dyschondroplasia. High calcium in the feed retards development of the lesion, whereas high phosphorus levels appear to accentuate the development of the lesions, as reported by Edwards and Veltmann, "The Role of Calcium and Phosphorus in the Etiology of Tibial Dyschondroplasia in Young Chicks," *J. Nutr.*, 113:1568 (1983).

Increases in the magnesium content of the chick diet decrease the incidence of tibial dyschondroplasia; however, the effect of magnesium is not as strong as that of calcium, as demonstrated by Edwards, "Studies on the Etiology of Tibial Dyschondroplasia in Chickens", *J. Nutr.*, 114:1001 (1984).

Given the large economic loss to meat producers caused by animals afflicted with tibial dyschondroplasia as well as the discomfort of the afflicted animal and the resulting unsanitary conditions caused by the diseased dysfunctional animal, it would be of great benefit to find an effective method and compositions to reduce the incidence of this disease.

U.S. Patent No. 5,154,925 discloses a method for treating tibial dyschondroplasia in fowl and other animals by administering to the animals a vitamin D_3 derivative including 1,25-dihydroxycholecalciferol; 1,25-dihydroxy-26,27-hexadeuterocholecalciferol; 1-hydroxy-cholecalciferol; 1,24,25-trihydroxychole-calciferol; 1,25-dihydroxy-24-fluorocholecalciferol; 25-hydroxycholecalciferol, or combinations thereof. The derivative is administered or fed to animals in a

pharmaceutical carrier including animal feed in a concentration between 0.10 and 20 micrograms of a vitamin D_3 derivative per kilogram of body weight per day.

It is therefore an object of the present invention to provide additional methods and compositions for the treatment and prevention of tibial dyschondroplasia in animals.

It is a further object of the present invention to provide methods and compositions for enhancing phytate phosphorus utilization in animals.

It is a further object of the present invention to provide a method and compositions for enhancing phytate phosphorus utilization while preventing or treating tibial dyschondroplasia.

It is a further object of the present invention to provide methods and compositions for decreasing phosphorus contamination of soil by animals.

It is a further object of the present invention to provide economical animal feed compositions containing reduced levels of supplemental calcium and phosphorus.

Summary of the Invention

The methods described herein include feeding animals a feed composition containing vitamin D, an effective amount of a hydroxylated metabolite, or derivative, of vitamin D_3, including 1,25-dihydroxycholecalciferol; 1,25-dihydroxy-26,27-hexadeuterocholecalciferol; 1-hydroxycholecalciferol; 1,24,25-trihydroxy-chole-calciferol; 1,25-dihydroxy-24-fluorocholecalciferol; 25-hydroxycholecalciferol; or combinations thereof; and active phytase, in animal feed. Feed compositions containing an amount of vitamin D_3 derivative effective for enhancing phytate phosphorus utilization and treatment or prevention of

tibial dyschondroplasia contain between 1 and 10 micrograms derivative p r kilogram feed.

It has been discovered that feeding a feed composition containing on or more of the above-defined 1-hydroxy vitamin D_3 derivatives and active phytase increases calcium utilization, so less dietary calcium is needed. A reduction in dietary calcium enhances phytate phosphorus utilization so that less supplemental phosphorus is required in the diet. Concurrent administration of phytase further enhances phytate phosphorus utilization.

For prevention of tibial dyschondroplasia, a feed composition containing between 1 and 10 micrograms of one or more of the above-defined hydroxylated vitamin D_3 derivatives per kilogram of feed is fed on the first day of life and is continued for at least three weeks. Treatment of tibial dyschondroplasia already established in poultry is achieved by feeding the feed composition as soon as the disease is discovered and continuing treatment until the animal is cured.

Brief Description of the Figures

Figure 1 is a drawing of the chemical structures of the synthesis of vitamin D_3 from 7-dehydro-cholesterol to previtamin D_3 to vitamin D_3 and the metabolism of vitamin D_3 to 1,25-dihydroxy-cholecalciferol.

Figure 2 is a drawing of the chemical structures of vitamin D_3 (cholecalciferol) (1), 1,25-dihydroxy-cholecalciferol (2), 1,25-dihydroxy-26,27-hexadeutero-cholecalciferol (3), 1-hydroxycholecalciferol (4), 1,24,25-trihydroxycholecalciferol (5), 1,25-dihydroxy-24-fluorocholecalciferol (6), and 25-hydroxy-chole-calciferol (7) as used in the methods and compositions described herein.

Figure 3 is a drawing of the chemical structure ,25-dihydroxycholecalciferol (8).

Detailed Description of the Invention

Methods and compositions for enhanced phytate horus utilization and the treatment and ntion of tibial dyschondroplasia in animals are ded herein. Enhancement of phytate phosphorus zation results in more economical food production ducing the need for dietary calcium and horus supplementation and reducing the amount of onmentally-hazardous phosphate excreted by the l. Treatment or prevention of tibial ondroplasia improves the health of the animals he sanitary conditions of the animal facility, llows more animals to be successfully processed re and after slaughter.

ncement of Phytate Phosphorus Utilization

A method for enhancement of phytate phosphorus ization is provided that includes feeding to als an animal feed composition containing an ctive amount of a 1-hydroxy vitamin D_3 derivative uding 1,25-dihydroxycholecalciferol (2), 1,25-droxy-26,27-hexadeuterocholecalciferol (3), 1-oxycholecalciferol (4), 1,24,25-trihydroxy-ecalciferol (5), 1,25-dihydroxy-24-rocholecalciferol (6), and combinations thereof.

Because the composition increases the lization of calcium from the diet, the diet, such an animal feed diet, contains lower levels of cium without risk of adverse consequences such as e deformity or breakage. Preferably, the total centration of calcium in the diet is between 0.5 1.3% by weight. Most preferably, the calcium centration is less than 1% by weight.

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calciferol (6), 25-hydroxycholecalciferol (7), and combinations th reof.

Preparation of Vitamin D_3 Derivatives

The vitamin D_3 derivatives described herein can be prepared by the following procedures, the teachings of which are incorporated by reference herein.

1,25-Dihydroxycholecalciferol can be prepared as described in *Biochemistry* 10(14), 2799 (1971), and U.S. Patents 4,310,467 and 3,697,559.

1,25-Dihydroxy-26,27-hexadeuterocholecalciferol can be prepared as described for the synthesis of 1,25-dihydroxycholecalciferol in *Tet. Let.* 40, 4147 (1972), with the substitution of a trideuteromethyl Grignard reagent in place of the methyl Grignard reagent used to add the carbons at the 26 and 27 positions.

1-Hydroxycholecalciferol can be prepared by the methods described in *J. Am. Chem. Soc.* 95(8), 2748 (1973) and U.S. Patent 3,741,996.

1,24,25-Trihydroxycholecalciferol can be prepared by the method described in U.S. Patent 3,847,955.

1,25-Dihydroxy-24-fluorocholecalciferol can be prepared by the procedure described in *J. Org. Chem.*, 53(5), 1040 (1988).

25-Hydroxycholecalciferol can be prepared as described in U.S. Patents 4,310,467 and 3,565,924.

Animals to Which the Composition is Administered

The methods and compositions described herein are particularly useful for enhancing phytate phosphorus utilization and reducing the incidence of tibial dyschondroplasia in chickens, especially broiler chickens and egg-laying chickens.

The methods and compositions are equally effective in enhancing phytate phosphorus utilization in other types of monogastric animals, including but not limited to humans, swin , and other fowl including

turkeys, pheasants, and ducks. The animals to which the animal feed containing the vitamin D_3 derivative is fed are generally not vitamin D-deficient, receiving an adequate supply of vitamin D in the diet and exposure to adequate amounts of sunlight.

Feeding of the above-described feed compositions according to the methods described herein are also effective in preventing or treating tibial dyschondroplasia by reducing or reversing the development of abnormal cartilage at the proximal end of the tibia in a variety of animals including but not limited to swine, dogs, rabbits, cattle, fish, and other fowl including turkeys, pheasants, and ducks. For example, Great Danes have a particular problem with tibial dyschondroplasia due to their rapid growth.

Effective Dosage Range and Method of Administration

An effective dosage of one or more of the vitamin D_3 derivatives described herein for either enhancement of phytate phosphorus utilization or prevention or treatment of tibial dyschondroplasia is between 0.1 and 20 micrograms per kilogram of body weight per day. However, it is inconvenient to weigh the animal and adjust and administer the dosage each day. It is more convenient and economical to prepare an animal feed or water composition containing a fixed amount of vitamin D_3 derivative. As the animal becomes larger, it will consume greater quantities of feed or water containing the vitamin D_3 derivative, thereby self-regulating administration of an appropriate dosage of the derivative.

For the foregoing reasons, the vitamin D_3 derivative is preferably fed to animals in an animal feed composition containing between 1 and 10 micrograms derivative per kilogram feed. A more preferred animal feed composition contains between 3 and 6 micrograms derivative per kilogram feed. Most

preferably, the animal feed composition is fed in feed containing approximately 5 micrograms vitamin D_3 derivative per kilogram feed. Dosages above 10 micrograms derivative per kilogram feed tend to cause a decreased animal growth rate.

It will be understood by those skilled in the art that the vitamin D_3 derivative could also be administered as a water composition wherein the concentration of derivative in the water is approximately one-half the concentration of derivative described above for use in feed because animals normally consume two volumes of water for every one volume of dry food. Therefore, the concentration of vitamin D_3 derivative in water is preferably between 0.5 and 5 micrograms per kilogram water.

Enhancement of both phytate phosphorus utilization and prevention of tibial dyschondroplasia can be achieved by feeding one or more of the vitamin D_3 derivatives in accordance with the methods described herein in animal feed from birth throughout the life of the animal. For example, in poultry, treatment is preferably begun at one day of age, and continued for at least three to four weeks to prevent the onset of tibial dyschondroplasia. Feeding the vitamin D_3 derivatives described herein to animals older than one day of age will continue to provide the desired therapeutic effect, but will be slightly less effective at preventing the onset of the tibial dyschondroplasia.

The compositions can also be fed in a feed compositions as a treatment for tibial dyschondroplasia already established in the animal. The same dosages are used as for prevention of the disease. The compositions should be fed to the animal as soon as the disease is discovered, and continued until the animal is cured. However, feeding the feed

compositions at this time will not reverse the abnormal shape of bones caused by the disease.

The effectiveness of feeding the composition on enhancement of phytate phosphorus utilization in poultry and other animals can be determined by measuring the phosphorus content of the feed and the phosphorus content of feces collected from the animals, and calculating the amount of phosphorus retained as described in the examples below.

The effectiveness of feeding the compositions on prevention or treatment of tibial dyschondroplasia in fowl can be determined by making a longitudinal cut across the tibia and inspecting it for incidence and severity of the disease, as described below in the examples.

The effectiveness of feeding the compositions containing the vitamin D_3 derivatives on prevention or treatment of dyschondroplasia in other animals can be determined by internal inspection of the bone for abnormal cartilage development or external inspection of bone for irregularities, bowed shape, or weakness.

Poultry Feed Composition for Phytate Phosphorus Enhancement

A preferred poultry feed composition for enhancement of phytate phosphorus utilization in poultry contains an effective amount of a 1-hydroxylated vitamin D_3 derivative such as 1,25-dihydroxycholecalciferol (2), 1,25-dihydroxy-26,27-hexadeuterocholecalciferol (3), 1-hydroxycholecalciferol (4), 1,24,25-trihydroxycholecalciferol (5), 1,25-dihydroxy-24-fluorocholecalciferol (6), or a combination thereof, in combination with sources of energy such as corn, fat, and soybean meal; sources of protein such as corn and soybean meal; mineral sources such as iodized sodium chloride; amino acids such as D,L-methionine; a vitamin mixture; a trace mineral

mixture; selenium concentrate (0.02% sodium selenite); and a calcium source such as limestone.

The vitamin mixture in the poultry or fowl feed composition provides in milligrams/kilogram of diet (except as noted) vitamin A (as all-trans-retinyl acetate), 5,500 IU; vitamin D_3 (cholecalciferol), 1100 ICU or 27.5 micrograms; vitamin E (all-rac-alpha-tocopheryl acetate), 11 IU; riboflavin, 4.4; calcium pantothenate, 12; nicotinic acid, 44; choline chloride, 220; vitamin B_{12}, 9 micrograms; vitamin B_6, 3.0; menadione (as menadione sodium bisulfite), 1.1; thiamin (as thiamin mononitrate) 2.2; folic acid, 3; biotin, 0.3; and ethoxyquin, 125.

The trace mineral mixture provides in milligrams per kilogram of diet MnO_2, 222; ZnO, 150, $FeSO_4$-$7H_2O$, 200; $FeCO_3$, 83; C_uSO_4, 29; and $Ca(IO_3)_2$, 15.

The vitamin D_3 derivative is preferably combined with the feed by first dissolving it in an alcohol, adding the solution to feed, and mixing the feed to evenly distribute the dissolved derivative.

A phytase solution is preferably added to the feed by first diluting the phytase with a powder, such as powdered soybean meal flour, and then mixing the diluted phytase with the feed composition containing the vitamin D_3 derivative. Alternatively, the phytase solution can be sprayed onto the feed and allowed to dry. Preferably, the feed contains between 30 and 1200 units of active phytase per kilogram feed. Active phytase is available commercially from Sigma Chemical Company, St. Louis, Mo.

The ingredients of a supplemented chicken feed to which the vitamin D_3 derivatives described herein may be added in the above-described dosages for enhanced phytate utilization in chickens and turkeys are described in Table 1. The amounts indicated are given in percent by weight. It will be understood by

those skilled in the art that the active vitamin D_3 derivatives described herein can also be administered in combination with other commercially formulated or similar feeds for chickens and other animals.

TABLE 1

Chicken Feed Composition for Phytate Phosphorus Enhancement

Ingredients	Amounts
Ground yellow corn	57.63
Soybean meal (dehulled)	35.00
Poultry fat (stabilized)	5.00
Iodized sodium chloride	0.45
D,L-Methionine (98%)	0.20
Vitamin premix (as described above)	0.25
Trace mineral premix (as described above)	0.10
Se concentrate (0.02% from sodium selenite)	0.05
Limestone	1.32

The vitamin D_3 derivatives described herein are efficacious for the enhancement of phytate phosphorus utilization when added to the above-described feed which has a calcium concentration of 0.5%. Broiler chickens are typically fed a diet which has a range of calcium of 0.7 to 1.4% by weight, while egg laying hens are fed a diet with a higher calcium level. This reduced level of calcium is achieved because the animal feed contains no dicalcium phosphate. Any calcium present in the feed is due to the presence of limestone.

A reduced level of phosphorus of 0.3% is also achieved by feeding the above-described animal feed lacking dicalcium phosphate. The phosphate present is due to phytate phosphorus alone.

<u>Poultry Feed Composition for Tibial Dyschondroplasia</u>

A preferred poultry feed composition for treatment or prevention of tibial dyschondroplasia in poultry contains an effective amount of a hydroxylated vitamin D_3 derivative such as 1,25-dihydroxycholecalciferol (2), 1,25-dihydroxy-26,27-hexadeuterocholecalciferol (3), 1-hydroxycholecalciferol (4), 1,24,25-trihydroxycholecalciferol (5), 1,25-dihydroxy-24-fluorocholecalciferol (6), 25-hydroxycalciferol (7), or a combination thereof, in combination with sources of energy such as corn, fat, and soybean meal; sources of protein such as corn and soybean meal; mineral sources such as iodized sodium chloride; amino acids such as D,L-methionine; a vitamin mixture; a trace mineral mixture; selenium concentrate (0.02% sodium selenite); a phosphorus source such as dicalcium phosphate; and calcium sources such as dicalcium phosphate and limestone.

The vitamin and trace mineral mixtures for the chicken feed composition for the treatment or prevention of tibial dyschondroplasia are the same as those set forth above for the chicken feed composition for enhancement of phytate phosphorus utilization.

The ingredients of a supplemented chicken feed to which the vitamin D_3 derivatives described herein may be added in the above-described dosages for treatment or prevention of tibial dyschondroplasia in chickens are described in Table 2. The amounts indicated are given in percent by weight. It will be understood by those skilled in the art that the active vitamin D_3 derivatives described herein can also be fed in combination with other commercially formulated or similar feeds for chickens and other animals.

TABLE 2

Chicken Fe d Compositi n f r Tibial Dyschondroplasia

Ingredients	Amounts
Ground yellow corn	56.81
Soybean meal (dehulled)	35.00
Poultry fat (stabilized)	5.00
Iodized sodium chloride	0.45
D,L-Methionine (98%)	0.20
Vitamin premix (as described above)	0.25
Trace mineral premix (as described above)	0.10
Se concentrate (0.02% from sodium selenite)	0.05
Dicalcium phosphate (feed grade)	1.86
Limestone	0.28

Chickens fed the diet described in Table 2 without the vitamin D_3 derivatives described herein will have a higher than normal incidence of tibial dyschondroplasia even though the diet contains a high level of vitamin D_3 because the diet has a high level of chloride and phosphorus and a low level of calcium. The average analyzed values for the chicken feed described in Table 2 are 0.32% chloride, 0.76% phosphorus, and 0.75% calcium.

The vitamin D_3 derivatives described herein are efficacious for the treatment or prevention of tibial dyschondroplasia in a feed having any calcium concentration, particularly those with a calcium composition of less than 2.5% by weight. However, a feed composition useful for both enhancement of phytate phosphorus utilization and prevention or treatment of tibial dyschondroplasia preferably has a calcium concentration of between 0.5 and 1.3% as described above.

A control system to test the effectiveness of the vitamin D_3 derivatives on reducing the incidence of tibial dyschondroplasia in fowl was developed using disulfiram [bis(diethylthiocarbamyl)disulfide], an

alcohol deterrent, in a dietary dosage of approximately 30 mg/kg feed per day. Disulfiram supplementation lowers the absorption of calcium when given as an oral dose compared to controls. It is known that thiruram [bis(dimethylthiocarbamyl)-disulfide], a compound used as a fungicide and bactericide which is structurally close to disulfiram and which also causes tibial dyschondroplasia, creates a rapid loss of intramuscularly-injected calcium immediately after dosing. This suggests that calcium in the blood and soft tissues (gastrointestinal tract) can be lost more easily from the birds receiving thiruram or disulfiram but that once the calcium goes into the bone both thiruram and disulfiram have little effect on turnover.

The present methods and compositions will be further understood with reference to the following non-limiting examples.

Example 1: Enhancement of Phytase Utilization with Vitamin D_3 Derivatives

The effectiveness of 1,25-dihydroxycholecalciferol (2), 1,25-dihydroxy-26,27-hexadeuterocholecalciferol (3), 1-hydroxycholecalciferol (4), 1,24,25-trihydroxycholecalciferol (5), and 1,25-dihydroxy-24-fluorocholecalciferol (6) in enhancing phytate phosphorus utilization in chickens has been demonstrated using the following experimental procedures.

Newly hatched Petersen X Arbor Acre cockerels obtained from a commercial hatchery were used. Animals were housed in electrically heated Petersime battery brooders with wire-mesh floors located in a room in which the temperature was maintained at 22°C, fluorescent lights were on 24 hours each day in the room and the cages, and sunlight was present during the day from large windows. No attempt was made to

limit the natural production of vitamin D_3. Feed and water were available to the chickens at all times.

Ten newly hatch d birds were randomly placed in each section of the Petersime batteries until the birds were nine days old. The measurements described below were calculated on a pen basis; all statistical analyses were conducted on pen averages. Blood samples were taken by cardiac puncture with heparinized syringes from one randomly chosen bird from each pen on the ninth day before weighing the birds. The blood samples were analyzed for plasma total calcium using Technicon Auto Analyzer Methodology set forth in a 1969 publication by the Technicon Instrument Co. Ltd. (Basingstoke, Hants, U.K.) and dialyzable phosphorus using Technicon Auto Analyzer Methodology set forth in a 1970 publication by the Technicon Instrument Co. Ltd.

The basal diet used in these studies is shown in Table 1 above. The basal diet contained 1100 ICU/kg (27.5 microgram/kg) of added D_3 and is low in calcium and phosphorus, having average analyzed values of 0.5% calcium and 0.3% phosphorus.

At nine days of age, pen body weights were obtained for all birds and their feed consumption was recorded. All birds were then killed by asphyxiation and examined at random for tibial dyschondroplasia and rickets by making a longitudinal cut across the tibia and scoring for incidence and severity of tibial dyschondroplasia in accordance with the method described in H. Edwards and J. Veltmann, "The Role of Calcium and Phosphorus in the Etiology of Tibial Dyschondroplasia in Young Chicks", *J. Nutr.*, 113, 1568 (1983). Briefly, using a three week old chicken as the reference animal, a score of zero indicates normal cartilage which is narrow with little irregularities. A score of one indicates cartilage which is thickened or shows considerable irregularities. A score of two

indicates that the cartilage is thickened and there is evidence of persisting prehypertrophic cartilage that is not calcified and which has not been invaded by vessels from the metaphysis. Deep irregularities are apparent. A score of three indicates a large mass of cartilage in the proximal end of the tibia. The birds were also scored for phosphorus deficient rickets at the same time they were scored for tibial dyschondroplasia. Chickens which had normal proliferating prehypertrophied zone and a lengthened metaphyseal primary spongiosa were classified as phosphorus deficient rickets. The left tibia was removed for bone ash determination on the fat-free bone.

In each experiment, the droppings from all of the chickens for the entire nine day experimental period were collected at the end of the experiment. They were dried in a force draft oven at 70°C, weighed, and ground in a Wiley Mill. Samples were then taken and, with a feed sample, analyzed for calcium by the fluorometric method of Hill, *Clin. Chem.* 2:122-130 (1955), phosphorus by the method of O'Neill and Webb, *J. Sci Food Agric.* 21:217-219 (1970), and phytate, or phytin, phosphorus by the method of Common, *J. Agric. Sci.* 30:113-131 (1940), the methods of which are incorporated by reference herein.

Phytate phosphorus in feed and feces samples was also determined by a modification of the method reported by Sandberg and Ahderinne, *J. Food Sci.* 51:547-550 (1986), the methods of which are incorporated by reference herein, for determination of phytate in feed and feed ingredients. The method has been modified and tested to allow high pressure liquid chromatography determination of phytate in poultry feces as follows. Pulverized dried excreta of 0.2 g (0.5 g for feed sample) was extracted using 20 ml 0.5

M HCl under vigorous mechanical agitation at room temperature for two hours. The extract was filtered through glass microfiber filter paper #934-AH (Whatman International, Maidstone, England) under vacuum. Four milliliters of the filtrate was then diluted with 16 ml of deionized water. The inositol phosphates were separated from the diluted filtrate solution and were concentrated using anion-exchange column chromatography. A glass column of 0.7 x 15 cm with a glass filter at the distal end was packed with 2 ml of anion-exchange resin (AG 1-x8, 200-400 mesh) (Bio-Rad, Richmond, CA) developed overnight in .025 M HCl.

The diluted filtrate solution of 20 ml was passed through the anion-exchange column at a flow rate of approximately 0.4 ml/min, the column was then washed with 4 ml 0.025 M HCl. Inositol phosphates were removed from the chromatographic column using 20 ml 2 M HCl. The eluate was collected in a 20 ml scintillation vial. An 8 ml fraction of eluent was freeze-dried.

The dried sample was then reconstituted with 0.8 ml of a filtered mobile phase consisting of 0.05 M formic acid:methanol (HPLC grade) 50:50, 1.5 ml/100 ml of tetrabutylammonium hydroxide (TBA-OH, 40% in water) (Sigma, St. Louis, MO) and 5 mM of ethylenediaminetetraacetic acid (EDTA). The pH of the mobile phase was adjusted to 4.3 by the addition of 6 M sulfuric acid. The mobile phase was filtered through a nylon membrane filter (0.45 micron pore size) under vacuum.

The analysis was performed using an HPLC pump, Eldex Model AA-72-S (Eldex, San Carlos, CA) equipped with a reverse phase C-18 Waters Radialpak column (Waters, Milford, MA) having a 5 mm inside diameter and 5 micron particle size. The inositol phosphates were detected by refractive index using a Waters Differential Refractometer, Model R401 (Waters,

Milford, MA). The optimal flow rate was 1.5 ml/min. Retention times and peak areas were measured by a Hewlett Packard Model 3390A integrator (Hewlett Packard, Palo Alto, CA). Samples were injected into a 20 microliter Rheodyne Model 7125 loop (Rheodyne, Cotati, CA). Sodium phytate was used as both the internal and external standard. The linearity of phytate concentration versus peak area was investigated by 20 microliter injections of solutions containing 5.6, 11.2, 16.8, and 22.4 micrograms of phytate.

In the first experiment, a sample containing a known amount of added hexaphosphoinositol was run with each sample to determine recovery. For the 24 fecal samples in this experiment, the recovery averaged 101.5%. Recovery studies were not conducted in later experiments and recovery was assumed to be 100%.

Experiment 1

Each of the twelve different dietary treatments described below were provided to two pens containing ten birds per pen.

1. One diet was a basal diet lacking supplemented inorganic phosphorus, phytase or the vitamin D_3 derivative $1,25-(OH)_2D_3$(2).

2. Three diets contained graded levels of phosphorus (0.1, 0.2 and 0.3%) from reagent grade $Na_2HPO_4H_2O$ which is indicated by the symbol P in the tables.

3. Four diets contained graded levels of phytase (Sigma Chemical Co., St. Louis, MO), at 75, 150, 300 and 600 units/kg of feed. The phytase was first mixed with powdered soybean meal flour, and the phytase and flour mixture was combined with the feed.

4. Four diets contained the four levels of phytase described above plus 10 micrograms/kg of $1,25-(OH)_2D_3$. The derivative was first diluted with

propylene glycol to a concentration of 10 micrograms per milliliter, and then the solution was mixed with feed in a Hobart mixer for 10 minutes.

All chickens were fed a corn-soybean diet containing only 0.67% calcium, 0.34% phosphorus, and a vitamin mixture supplying 1100 ICU D_3/kg of diet.

The results are shown in Tables 3A, 3B and 3C below.

Analysis of Experiment 1 Results

The birds fed the basal phosphorus-deficient diet had very low nine day weights and gain to feed ratios and developed very severe phosphorus deficient rickets. The bone ash value of 22.9% ash indicates extremely poor bone calcification. Fifty percent of the dietary phytate appeared in the feces as hexa, hepta and tetra phosphoinositol, (IP6, IP5 and IP4) indicating that only half of the phytate phosphorus in the basal diet was available to the chickens.

The addition of phosphorus to the diet resulted in increased nine day weights, gain:feed ratios, blood plasma phosphorus and bone ash. The incidence of phosphorus-deficient rickets was decreased. The amount of phosphorus in the feces was approximately the same.

The addition of phytase to the diet resulted in an increase in nine day weight and a small decrease in the amount of hexaphosphoinositol in the feces. There was no significant increase in bone ash. The calculated phytate retention value was 60.7% for birds fed the highest level of phytase.

The animals given feed containing 1,25-$(OH)_2D_3$(2) and phytase had higher nine day body weights than those fed the basal diet. The addition of 1,25-$(OH)_2D_3$ and phytase to the diet resulted in increased bone ash values and a significant decrease in the levels of hexa and penta phosphoinositol in the feces as compared to all other treatments. The level of

tetraphosphoinositol was so low in feces that it was not detectable with the method used. The calculated retention data indicate that th se animals utilized from 76 to 88% of the phytate phosphorus consumed as compared to 50% by the animals fed the basal diet and 52 to 60% by the animals fed the basal diet plus phytase alone.

The addition of 1,25-$(OH)_2D_3$ caused a large increase in the utilization of phytate phosphorus as indicated from the bone ash values and amounts of phytate excreted in the feces.

TABLE 3A: Effect of dietary phosphorus levels and phytase and/or 1,25-$(OH)_2D_3$ supplementation on growth, development of tibial dyschondroplasia and Ca, P, and phytate phosphorus retention by broilers

Treatments	Wt. 9-d	Gain: feed	Bone ash	Tibial dyschondroplasia Incidence	Tibial dyschondroplasia Score	Tibial dyschondroplasia #3 Scores	Phosphorus rickets Incidence	Retention by the broiler Calcium	Retention by the broiler Phosphorus	Retention by the broiler Phytate Phosphorus[1]
	g		%	%		%	%	%	%	%
Basal 0% P	133	.672	22.9	12	2.00	7	41	49.53	46.47	50.50
.1% P	168	.753	21.8	35	1.80	10	5	52.76	40.72	46.51
.2% P	164	.701	36.5	15	.67	0	0	60.87	28.56	67.78
.3% P	163	.701	38.5	5	.50	0	0	62.20	9.50	50.72
75 units Phytase	144	.724	23.9	0	0	0	33	47.06	47.20	56.36
150 units Phytase	148	.716	24.7	5	.50	0	28	44.80	53.55	59.06
300 units Phytase	142	.707	24.4	0	0	0	40	42.62	49.04	52.67
600 units Phytase	153	.733	26.5	25	1.63	10	30	43.54	55.39	64.85

TABLE 3A CONT.

Treatments	Wt. 9-d	Gain: feed	Bone ash	Tibial dyschondroplasia Incidence	Tibial dyschondroplasia Score	Tibial dyschondroplasia #3 Scores	Phosphorus rickets Incidence	Retention by the broiler Calcium	Retention by the broiler Phosphorus	Retention by the broiler Phytate Phosphorus[1]
	g		%	%		%	%	%	%	%
75 units P + 10 μg/kg 1,25-$(OH)_2D_3$	162	.727	31.7	0	0	0	5	51.14	61.73	80.60
150 units P + 10 μg/kg 1,25-$(OH)_2D_3$	152	.700	32.1	5	.50	0	0	51.15	67.24	87.41
300 units P + 10 μg/kg 1,25-$(OH)_2D_3$	153	.690	33.5	5	.50	0	5	56.80	65.50	83.36
600 units P + 10 μg/kg 1,25-$(OH)_2D_3$	160	.729	34.0	5	.50	0	0	53.50	67.31	82.23
Mean ± SEM	153 ± 5	.713 ± .016	30.0 ± .9	9 ± 8	.72 ± .51	2 ± 3	15 ± 8	51.33 ± 3.29	49.35 ± 1.60	65.17 ± 3.99

[1]Total feed and fecal phytate phosphorus determined by the ferric chloride method.

TABLE 3B: Effect of dietary phosphorus levels and phytase and/or 1,25-$(OH)_2D_3$ supplementation on blood Ca and P,phytin phosphorus excretion, and calculated retention of phytate phosphorus

Treatments	Blood plasma		Phytin phosphorus excretion expressed as percent of total phytin phosphrus ingested				Phytin Phosphours retained
	Calcium	Dialyzable phosphorus	IP4	IP5	IP6	Total Excreted	(100 - total excreted)
	mg/kg	mg/kg	%	%	%	%	%
Basal 0% P	136	20.00	.25	5.20	44.83	50.4	49.8
.1% P	142	20.50	.71	5.76	46.92	53.4	46.7
.2% P	112	59.50	.81	2.70	37.06	40.6	59.5
.3% P	105	75.00	1.46	6.87	46.00	54.4	45.9
75 units Phytase	137	15.80	.28	5.05	43.12	48.4	51.6
150 units Phytase	145	14.80	.00	4.23	37.46	41.7	58.5
300 units Phytase	161.	7.67	.39	5.14	40.66	46.2	53.8
600 units Phytase	145	1.83	.43	4.48	34.46	39.4	60.7

TABLE 3B (CONT.)

Treatments	Blood plasma		Phytin phosphorus excretion expressed as percent of total phytin phosphrus ingested				Phytin Phosphours retained (100 - total excreted)
	Calcium	Dialyzable phosphorus	IP4	IP5	IP6	Total Excreted	
	mg/kg	mg/kg	%	%	%	%	%
5 units P + 10 μg/kg 1,25-$(OH)_2D_3$	168	18.0	.00	1.28	22.43	23.8	76.3
150 units P + 10 μg/kg 1,25-$(OH)_2D_3$	178	14.0	.00	1.12	19.53	20.7	79.4
300 units P + 10 μg/kg 1,25-$(OH)_2D_3$	157	24.0	.000	.61	12.91	13.5	86.5
600 units P + 10 μg/kg 1,25-$(OH)_2D_3$	148	25.0	.000	.13	12.07	12.3	87.8
Mean ± SEM	144 ± 14	24.7 ± 6.6	.36 ± .05	3.55 .75	33.12 ± 2.54	37.0 3.1	63.0 ± 3.1

TABLE 3C: Phytate content of fecal samples, determined by the ferric chloride and HPLC procedures and calculated retention of phytate phosphorus by each procedure

Treatments	Food[1] consumed	Feces excreted	Phytate Phosphorus in feces by $FeCl^3$	Phytate phosphorus in feces by HPLC procedure				Phytate phosphorus retention	
				IP4	IP5	IP6	Total	$FeCl^3$	HPLC
	g	g	mg/g	mg/g	mg/g	mg/g	mg/g		
Basal 0% P	1345	402	3.53	.025	.490	4.23	4.75	51	50
.1% P	1660	451	3.50	.060	.490	3.99	4.54	47	47
.2% P	1725	602	1.97	.065	.230	3.03	3.32	68	60
.3% P	1715	606	2.98	.115	.550	3.68	4.35	51	46
75 units Phytase	1395	460	2.87	.025	.435	3.73	4.19	56	52
150 units Phytase	1305	434	2.63	.000	.360	3.18	3.54	59	59
300 units Phytase	1385	483	2.93	.030	.410	3.23	3.68	53	54
600 units Phytase	1500	491	2.30	.035	.385	2.96	3.38	65	61

TABLE 3C (CONT)

Treatments	Food[1] consumed	Feces excreted	Phytate Phosphorus in feces by FeCl[3]	Phytate phosphorus in feces by HPLC procedure				Phytate phosphorus retention	
				IP4	IP5	IP6	Total	FeCl[3]	HPLC
	g	g	mg/g	mg/g	mg/g	mg/g	mg/g		
75 units P + 10 μg/kg 1,25-$(OH)_2D_3$	475	519	1.17	.000	.105	1.80	1.90	81	76
150 units P + 10 μg/kg 1,25-$(OH)_2D_3$	1565	564	0.76	.000	.090	1.53	1.62	87	79
300 units P + 10 μg/kg 1,25-$(OH)_2D_3$	1510	522	1.03	.000	.050	1.06	1.11	83	87
600 units P + 10 μg/kg 1,25-$(OH)_2D_3$	1610	561	1.09	.000	.010	0.98	0.99	82	88
Mean ± SEM	1515 ± 96	508 ± 50	2.23 ± .28	.030 ± .015	.300 ± .067	2.78 ± .27	3.11 ± .33		

Experiment 2

Each of the eight different dietary treatments set forth below were provided to three pens containing ten birds per pen.

1. One diet was a basal diet lacking supplemental inorganic phosphorus, phytase or the vitamin D_3 derivative 1,25-$(OH)_2D_3$ (2).

2. One diet contained 0.2% supplemental phosphorus($Na_2HPO_4H_2O$) alone.

3. One diet contained 75 units of phytase alone.

4. One diet contained 5 micrograms/kg 1,25-$(OH)_2D_3$ alone.

5. One diet contained both 0.2% supplemental phosphorus plus 75 units of phytase.

6. One diet contained both 0.2% supplemental phosphorus plus 5 micrograms/kg 1,25-$(OH)_2D_3$.

7. One diet contained both 75 units of phytase plus 5 micrograms/kg 1,25-$(OH)_2D_3$.

8. One diet contained 0.2% supplemental phosphorus plus 75 units of phytase plus 5 micrograms/kg 1,25-$(OH)_2D_3$.

All chickens were fed a corn-soybean diet containing only 0.6% calcium, 0.34% phosphorus, and a vitamin mixture supplying 1100 ICU D_3/kg of diet.

The results are shown in Table 4 below.

Analysis of Experiment 2 Results

The addition of 1,25-$(OH)_2D_3$ (2) to the diet caused an increase in the utilization of phytate phosphorus by the broiler chickens from 31% to 68%. The combination of phytase and 1,25-$(OH)_2D_3$ raised the utilization to 79%.

TABLE 4: Effect of 1,25-$(OH)_2D_3$ plus phytase

Treatments	Wt. 9-d	Gain: feed	Bone ash	Tibial dyschondroplasia Incidence	Tibial dyschondroplasia Score	Tibial dyschondroplasia #3 scores	Phos. rickets incidence	Blood Plasma Ca	Blood Plasma Dialyzable P	Retention by the broiler Ca	Retention by the broiler P	Retention by the broiler Phytate P	Metabolizable energy f diet
	g		%	%		%		mg/kg	mg/kg	%	%	%	kcal/kg
Basal 0% P	145	.732	24.2	7	0.67	0	55	133.5	12.2	29.8	43.6	31.3	2.88
.2% P	170	.721	34.8	10	1.00	3	0	112.1	50.3	51.5	49.5	42.3	2.79
75 units phytase	154	.748	23.7	7	0.67	0	60	126.0	11.9	30.6	44.1	41.6	2.98
5 μg/kg 1,25-$(OH)_2D_3$	172	.760	29.8	17	1.00	0	10	136.5	14.5	44.8	64.8	68.4	2.99
.2% P + 75 units phytase	181	.772	35.4	7	1.33	3	0	118.3	61.3	49.3	51.6	44.4	2.82
.2% P + 5 μg/kg 1,25-$(OH)_2D_3$	186	.776	35.9	3	1.00	3	0	116.2	61.7	57.3	52.8	62.5	2.85

TABLE 4 (CONT)

Treatments	Wt. 9-d	Gain: feed	Bone ash	Tibial dyschondroplasia Incidence	Tibial dyschondroplasia Score	Tibial dyschondroplasia #3 scores	Phos. rickets incidence	Blood Plasma Ca	Blood Plasma Dialyzable P	Retention by the broiler Ca	Retention by the broiler P	Retention by the broiler Phytate P	Metabolizable energy of diet
	g		%	%		%		mg/kg	mg/kg	%	%	%	kcal/kg
75 units Phytase + 5 μg/kg 1,25-$(OH)_2D_3$	167	.735	30.1	17	1.33	3	27	119.6	39.7	46.2	59.5	79.4	2.84
.2% P + 75 units phytase + 5 μg/kg 1,25-$(OH)_2D_3$	178	.772	35.8	10	1.67	3	7	128.5	66.2	53.0	53.7	67.7	2.87
Mean ± SEM	169± 4	.752± .016	31.2± .6	10± .06	1.08± .60	2.08± 2.64	20± 6	123.8± 6.1	39.7± 8.7	45.3± 3.4	52.1± 2.4	54.7± 4.5	2.88± .03

Experiment 3

Each of the twelve different dietary treatments described below were provided to two pens containing ten birds per pen.

1. One diet was a basal diet lacking supplemental phytase or 1,25-$(OH)_2D_3$ (2).

2. Five diets contained graded levels of phytase from 37.5 units to 600 units.

3. One diet contained basal phytase plus 5 microgram/kg feed of 1,25-$(OH)_2D_3$.

4. Five diets contained graded levels of phytase plus 5 microgram/kg feed of 1,25-$(OH)_2D_3$.

The results are shown in Table 5 below. In Table 5, and subsequent tables, the abbreviations TDI and TDS represent tibial dyschondroplasia incidence and tibial dyschondroplasia score, respectively. N3 indicates the number 3 scores for tibial dyschondroplasia. Ri is an abbreviation for rickets incidence. B.A. is an abbreviation for bone ash. CAR, PR, and PPR represent calcium retention, phosphorus retention and phytate phosphorus retention respectively.

Analysis of Experiment 3 Results

The addition of 1,25-$(OH)_2D_3$ (2) to the diet caused an increase in the utilization of phytate phosphorus by the broiler chickens which was apparent from growth, bone ash, blood calcium, and total phosphorus retention. The addition of 1,25-$(OH)_2D_3$ in the presence of graded levels of phytase resulted in a gradual increase in the phytate phosphorus utilization from 67% when no phytase was present to 89% when 600 units of phytase/kg of feed was present in the diet.

TABLE 5: Effect of $1,25-(OH)_2D_3$ plus graded levels of phytase

Treatments	9-d wt.	Tibial dyschondroplasia TDI	TDS	N3	Ri	G:F	B.A.	Blood plasma Ca	Dialyzable P	Survivors	CAR	PR	PPR
	g	%		%	%	/	%	mg/kg	mg/kg	%	%		%
Basal	147	20	2.00	6	62	.692	23.7	9.83	4.70	16/20	30.87	41.58	48.63
37.5 units Phytase	172	19	1.00	0	32	.724	26.6	11.83	2.50	16/20	36.28	43.61	66.38
75 units Phytase	161	0	.00	0	60	.715	23.3	12.10	1.70	20/20	26.60	37.88	49.68
150 units Phytase	182	10	.75	0	70	.768	24.0	13.57	.75	20/20	17.63	33.92	52.01
300 units Phytase	182	5	.50	0	60	.789	24.3	12.63	1.20	20/20	17.83	42.39	54.15
600 units Phytase	185	0	.00	0	30	.764	26.4	13.38	.85	20/20	19.83	47.08	73.31
Basal + 5 μg $1,25-(OH)_2D_3$	187	10	.50	0	40	.808	26.9	14.30	1.15	18/20	31.14	55.89	66.59
37.5 Phytase + 5 μg $1,25-(OH)_2D_3$	184	15	2.00	5	25	.773	29.9	14.92	.95	20/20	37.28	57.03	79.11

TABLE 5 (CONT)

Treatments	9-d wt.	Tibial dyschondroplasia TDI	TDS	N3	Ri	G:F	B.A.	Blood plasma Ca	Dialyzable P	Survivors	CAR	PR	PPR
	g	%		%	%		%	mg/kg	mg/kg	%	%		%
75 Phytase + 5 μg 1,25-$(OH)_2D_3$	181	0	.00	0	25	.776	28.7	13.60	1.70	20/20	35.73	57.74	80.07
150 Phytase + 5 μg 1,25-$(OH)_2D_3$	189	6	.50	0	36	.752	28.4	14.43	.95	19/20	34.91	56.90	84.09
300 Phytase + 5 μg 1,25-$(OH)_2D_3$	194	25	2.00	5	20	.788	30.1	12.99	.78	20/20	42.87	61.41	84.89
600 Phytase + 5 μg 1,25-$(OH)_2D_3$	187	21	1.00	0	11	.766	32.5	14.50	1.75	19/20	44.09	61.25	88.74
Mean ± SEM	179 ± 4	11 ± 7	.85 ± .60	1 ± 3	39 ± 10	.760 ± .015	27.1 ± .7	13.2 ± 1.0	1.58 ± .64		31.25 ± 4.89	49.71 ± 3.77	68.97 ± 7.90

Experiment 4

The objective of experiment 4 was to determine if the addition of 1,25-$(OH)_2D_3$ to a corn-soybean diet containing 0.6% calcium would enhance the utilization of phytate phosphorus in the presence of various levels of phosphate.

Each of the twelve different dietary treatments set forth below were provided to three pens containing ten birds per pen.

1. One diet was a basal diet lacking supplemental inorganic phosphorus, phytase or 1,25-$(OH)_2D_3$ (2).

2. Two diets contained graded levels of phosphorus (0.1 and 0.2%) from reagent grade $Na_2HPO_4H_2O$.

3. One diet contained basal phosphate plus 150 units of phytase.

4. Two diets contained graded levels of phosphorus (0.1 and 0.2%) plus 150 units of phytase (Sigma Chemical Co., St. Louis, MO).

5. One diet contained basal levels of phosphorus plus 5 microgram/kg feed of 1,25-$(OH)_2D_3$.

6. Two diets contained graded levels of phosphorus (0.1 and 0.2%) plus 5 microgram/kg feed of 1,25-$(OH)_2D_3$.

7. One diet contained basal levels of phosphorus plus phytase plus 5 micrograms/kg 1,25-$(OH)_2D_3$.

8. Two diets contained graded levels of phosphorus (0.1 and 0.2%) plus 150 units of phytase plus 5 microgram/kg feed of 1,25-$(OH)_2D_3$.

All chickens were fed a corn-soybean diet containing only 0.6% calcium, 0.34% phosphorus, and a vitamin mixture supplying 1100 ICU D_3/kg of diet.

The results are shown in Table 6 below.

Analysis of Experiment 4 Results

The addition of inorganic phosphate to the diet decreases phytate phosphorus utilization and decreases the effectiveness of $1,25\text{-}(OH)_2D_3$ (2) in increasing phytate phosphorus utilization.

TABLE 6: Effects of phosphorus, phytase and 1,25-$(OH)_2D_3$

Diets	9-d wt.	TDI	TDS	N3	Ri	RiS	Ri#3	G:F	B.A.	TCa	DP	Survivors	CAR	PR	PPR
	g	%		%	%		%		%	%	%				
Basal	153	13	1.00	6	75	3.00	7	.693	24.3	12.33	1.22	18/20	39.02	49.04	53.89
.1% P	187	0	0.00	0	50	3.00	50	.769	31.8	11.95	1.53	20/20	30.08	53.18	53.63
.2% P	202	22	3.00	22	35	3.00	38	.784	34.2	10.60	4.22	19/20	53.77	54.25	48.02
150 units Phytase conc.	188	10	1.00	5	90	3.00	90	.801	24.7	13.37	.70	20/20	35.09	50.07	63.49
.1% P + 150 units Phytase conc.	193	25	1.30	20	30	3.00	30	.771	32.1	10.29	2.74	20/20	45.17[abc]	57.48	58.86
.2% P + 150 units Phytase conc.	203	11	1.50	11	6	1.50	6	.804	34.8	11.13	4.37	19/20	53.10[ab]	53.23	51.36
Basal + 5 μg/kg 1,25-$(OH)_2D_3$	187	0	0.00	0	40	3.00	40	.732	30.8	13.88	1.19	20/20	48.93[abc]	65.89	79.40
.1% P + 5 μg/kg 1,25-$(OH)_2D_3$	193	5	1.50	5	10	3.00	10	.795	34.3	13.23	3.02	20/20	51.16	62.64	72.16
.2% P + 5 μg/kg 1,25-$(OH)_2D_3$	198	16	3.00	16	15	3.00	15	.817	35.8	11.33	4.73	19/20	55.69	57.72	72.66

TABLE 6 (CONT)

Diets	9-d wt.	TDI	TDS	N3	Ri	RiS	Ri#3	G:F	B.A.	TCa	DP	Survivors	CAR	PR	PPR
	g	%		%	%		%		%	%	%				
Basal + 150 units Phytase + 5 μg/kg 1,25-$(OH)_2D_3$	181	10	3.00	10	25	3.00	25	.743	31.6	11.98	2.43	20/20	50.17	66.11	76.64
.1% P + 150 units Phytase + 5 μg/kg 1,25-$(OH)_2D_3$	199	10	2.00	5	5	1.50	5	.810	35.6	11.68	3.36	20/20	56.02	62.63	75.02
.2% P + 150 units Phytase + 5 μg/kg 1,25-$(OH)_2D_3$	182	0	0.00	0	5	1.50	5	.709	36.1	10.65	4.54	20/20	54.44	49.08	63.85
Mean ± SEM	188± 6	10± 10	1.44± .88	8± 8	32± 16	2.63± .75	32± 17	.769± .020	32.2± .8	11.87± .06	2.83± .45		47.72± 5.90	56.77± 2.63	64.08± 7.176

Experiment 5

The objective of experim nt 5 was to determine if the addition of 1,25-$(OH)_2D_3$ (2) to a corn-soybean meal diet with three levels of calcium with and without added phytase would enhance the utilization of phytate phosphorus.

Each of the twelve different dietary treatments set forth below were provided to three pens containing ten birds per pen.

1. Three diets contained graded levels of calcium (0.40%, 0.57% and 0.72%). The concentration of 0.40% calcium was obtained by adding 17.6 milligrams limestone per kilograms feed. The concentration of 0.57% calcium was obtained by adding 35.2 mg/kg limestone. The 0.72% calcium value was obtained by adding 52.8 mg/kg limestone.

2. Three diets contained 75 units phytase per kilogram feed plus each of the graded levels of calcium.

3. Three diets contained 5 microgram/kg feed of 1,25-$(OH)_2D_3$ plus each of the graded levels of calcium.

4. Three diets contained 5 microgram/kg feed of 1,25-$(OH)_2D_3$ plus phytase plus the three graded levels of calcium.

All chickens were fed a corn-soybean diet containing the diet specified above and a vitamin mixture supplying 1100 ICU D_3/kg of diet.

The results are shown in Table 7 below.

Analysis of Experiment 5 Results

Feeding the low calcium diet (0.40%) made it possible to obtain a very high level of phytate phosphorus retention (86%) with the basal diet containing no added phytase or 1,25-$(OH)_2D_3$ (2) to the diet. The addition of phytase or 1,25-$(OH)_2D_3$ to the diet resulted in slightly greater phytate utilization.

However, greater phytate utilization was apparent when both phytase and 1,25-$(OH)_2D_3$ were added to the diet, especially in the higher dietary calcium levels.

TABLE 7: Effect of 1,25-$(OH)_2D_3$ and graded levels of calcium

Treatments	9-d wt.	TDI	TDS	N3	Ri	RiS	Ri#3	G:F	B.A.	TCa	DP	Survivors	CAR	PR	PPR
	g	%		%	%		%		%	%	%		%	%	%
0.40% Calcium	177	5	1.50	5	90	3.00	90	.765	28.1	8.18	7.90	19/20	68.61	37.66	86.21
0.57% Calcium	207	15	1.50	5	90	2.11	50	.806	32.8	9.43	8.15	19/20	60.57	49.07	78.39
0.72% Calcium	192	15	1.25	5	65	1.91	30	.780	34.2	10.60	4.70	20/20	51.51	52.01	55.16
0.40% Calcium + Phytase	189	5	1.50	5	95	2.80	85	.825	28.5	8.63	9.30	20/20	62.27	46.34	89.37
0.57% Calcium + Phytase	201	15	3.00	15	60	2.12	35	.827	33.3	9.88	7.10	20/20	58.79	52.56	69.95
0.72% Calcium + Phytase	200	37	2.42	26	64	2.75	53	.797	35.7	11.25	6.95	19/20	52.40	54.84	63.38
0.40% Calcium + 1,25-$(OH)_2D_3$	185	15	1.50	5	75	2.45	55	.732	30.9	9.33	7.45	20/20	64.53	39.67	94.42
0.57% Calcium + 1,25-$(OH)_2D_3$	211	21	2.25	16	70	2.40	42	.831	34.8	10.58	7.30	19/20	64.07	53.27	78.67

TABLE 7 (CONT)

Treatments	9-d wt.	TDI	TDS	N3	Ri	RiS	Ri#3	G:F	B.A.	TCa	DP	Survivors	CAR	PR	PPR
	g	%		%	%		%		%	%	%		%	%	%
1.89% Calcium[1] + 1,25-$(OH)_2D_3$	160	0	0.00	0	16	1.50	6	.759	34.1	13.30	4.60	19/20	50.40	47.46	37.84
0.40% Calcium + 1,25-$(OH)_2D_3$ + Phytase	190	20	2.50	15	85	2.75	75	.764	31.1	9.73	7.70	20/20	71.88	40.91	90.87
0.57% Calcium + 1,25-$(OH)_2D_3$ + Phytase	185	15	0.50	0	70	1.25	10	.716	35.3	10.35	8.25	20/20	59.73	44.49	96.70
0.72% Calcium + 1,25-$(OH)_2D_3$ + Phytase	191	22	2.00	11	28	1.84	11	.786	36.7	11.10	7.90	20/20	63.00	56.63	75.51
Mean ± SEM	191 ± 7	15 ± 6	1.66 ± .72	9 ± 4	67 ± 9	2.24 ± .26	45 ± 9	.782 ± .029	33.0 ± .6	10.20 .71	7.28 ± .80	60.64 ±	48.07 ± 2.52	76.20 ± 3.90	2.74

[1]There was a mixing error in preparing the diet that should have contained 0.72% calcium and 1,25-$(OH)_2D_3$. The diet mixed and fed actually contained 1.89% calcium.

Experiment 6

The objective of experiment 6 was to determine if the addition of the vitamin D_3 derivatives 1,25-$(OH)_2D_3$ (2), 1-OHD_3 (4) or 24,25-$(OH)_2D_3$ (8) to a corn-soybean meal diet with and without added phytase would enhance the utilization of phytate phosphorus.

Each of the eight different dietary treatments set forth below were provided to two pens containing ten birds per pen.

1. One diet contained basal levels of phytase.

2. One diet contained 150 units of phytase.

3. Three diets contained 5 micrograms/kg of either 1,25-$(OH)_2D_3$ (2), 1-OHD_3 (4), or 24,25-$(OH)_2D_3$ (8).

4. Three diets contained 150 units of phytase plus 5 micrograms/kg of either 1,25-$(OH)_2D_3$ (2), 1-OHD_3 (4), or 24,25-$(OH)_2D_3$ (8).

All chickens were fed a corn-soybean diet containing the diet specified above and a vitamin mixture supplying 1100 ICU D_3/kg of diet.

The results are shown in Table 8 below.

Analysis of Experiment 6 Results

The 1,25-$(OH)_2D_3$ (2) and 1-OHD_3 (4) supplementation of the diet resulted in increased nine-day weights and bone ash as contrasted to supplementation with 24,25-$(OH)_2D_3$ (8) which had no effect on these criteria in the presence or absence of supplemental phytase. In this study, the 1-OHD_3 (4) and 24,25-$(OH)_2D_3$ (8) did not appear to increase phytate phosphorus utilization in the absence of phytase. Supplementation with 1,25-$(OH)_2D_3$ (2) increased phytate phosphorus utilization in the presence or absence of phytase.

TABLE 8: Effects of 1,25-$(OH)_2D_3$, 1-OHD_3, and 24,25-$(OH)_2D_3$

Treat-ments	9-d wt.	TDI	TDS	TD N3	Ri I	Ri S	Ri #3	G:F	B.A.	TCa	DP	CAR	PR	PPR
	g	%			%				%	%	%			
Basal	143	7	1.33	3	86	3.00	86	.655	24.3	11.92	1.63	40.29	49.18	61.11
150 Phytase	159	8	1.33	4	82	3.00	79	.685	24.9	13.25	.54	37.49	47.47	57.18
150 Phytase + 1,25-$(OH)_2D_3$	166	17	1.11	7	53	3.00	53	.704	29.6	15.63	1.27	40.53	62.28	80.29
150 Phytase + 1-OHD_3	168	17	2.11	7	58	3.00	59	.741	28.1	14.47	.67	41.76	53.61	69.37
150 Phytase + 24,25-$(OH)_2D_3$	152	7	.67	4	75	3.00	82	.716	24.3	11.77	2.07	33.32	47.05	69.17
1,25-$(OH)_2D_3$	168	4	1.00	4	24	3.00	25	.705	31.7	12.50	1.93	54.57	65.27	72.93
1-OHD_3	160	10	.67	0	77	3.00	77	.718	26.1	14.77	.86	37.15	49.67	61.29
24,25-$(OH)_2D_3$	150	4	1.00	4	72	3.00	71	.707	23.5	14.84	.61	30.99	49.65	62.45
Mean ± SEM	158± 4	9± 6	1.11± .80	4± 4	66± 8	3.00± 0	66± 8	.704± .023	26.6± .4	13.61± .89	1.20± .39	39.57± 2.44	53.21± 2.41	67.07± 7.74

Experiment 7

The objective of experiment 7 was to determine if the addition of the vitamin D_3 derivatives 1,24,25-$(OH)_3D_3$ (5), 1,25-$(OH)_2$-24-FD_3 (6), or 1,25-$(OH)_2$-26,27[$^3H]_6D_3$ (3) to a corn-soybean meal diet with and without added phytase would enhance the utilization of phytate phosphorus.

Each of the eight different dietary treatments set forth below were provided to two pens containing ten birds per pen.

1. One diet contained basal levels of phytase and vitamin D_3 derivatives.

2. One diet contained 150 units of phytase.

3. Three diets contained 5 micrograms/kg of either 1,24,25-$(OH)_3D_3$ (5), 1,25-$(OH)_2$-24-FD_3 (6) or 1,25-$(OH)_2$-26,27[$^3H]_6D_3$ (3).

4. Three diets contained 150 units of phytase plus 5 micrograms/kg of either 1,24,25-$(OH)_3D_3$ (5), 1,25-$(OH)_2$-24-FD_3 (6) or 1,25-$(OH)_2$-26,27[$^3H]_6D_3$ (3).

All chickens were fed a corn-soybean diet containing the diet specified above and a vitamin mixture supplying 1100 ICU D_3/kg of diet.

The results are shown in Table 9 below.

Analysis of Experiment 7 Results

All three of the D_3 derivatives increased the utilization of phytate phosphorus by the broiler chickens, but 1,25-$(OH)_2$-26,27[$^3H]_6D_3$ (3) was more effective than the other two vitamin D_3 derivatives.

TABLE 9: Effects of 1,24,25-$(OH)_3D_3$, 1,25-$(OH)_2$-24-FD_3, and 1,25-$(OH)_2$-26,27[$^3H]_6D_3$

Treat-ments	9-d wt.	TDI	TDS	TD N3	Ri I	Ri S	Ri #3	G:F	B.A.	TCa	DP	CAR	PR	PPR
	g	%			%				%	%	%			
Basal	155	10	1.00	3	53	2.90	50	.703	25.79	10.38	1.37	40.12	46.32	53.11
150 units Phytase	158	10	1.00	0	73	3.00	73	.728	24.19	10.92	.31	39.73	53.96	63.61
Phytase + 1,24,25-$(OH)_3D_3$	168	7	.66	0	90	2.92	87	.750	25.78	11.18	.21	43.65	55.93	64.53
Phytase + 1,25-$(OH)_2$-24R-FD_3	161	18	1.00	0	65	2.89	61	.700	27.04	10.67	.64	44.66	56.98	66.85
Phytase + 1,25-$(OH)_2$-26,27 [$^3H]_6D_3$	169	7	.33	0	37	3.00	37	.749	31.00	10.55	2.40	50.65	70.09	81.47
1,24,25-$(OH)_3D_3$	151	7	.33	0	79	2.93	76	.680	26.27	10.58	.44	46.90	54.68	67.71
1,25-$(OH)_2$-24R-FD_3	162	18	1.66	4	72	3.00	72	.737	27.78	10.68	.74	47.36	53.39	63.12
1,25-$(OH)_2$-26,27 [$^3H]_6D_3$	160	3	.33	0	30	3.00	30	.716	30.25	10.12	1.57	48.87	62.08	77.03

TABLE 9 (CONT.)

Treat-ments	9-d wt.	TDI	TDS	TD N3	Ri I	Ri S	Ri #3	G:F	B.A.	TCa	DP	CAR	PR	PPR
	g	%			%				%	%	%			
Mean ± SEM	160 ± 5	10 ± .6	.83 ± .41	1 ± 2	63 ± 9	2.95 ± .07	61 ± 9	.720 ± .017	27.26 ± .64	10.64 ± .64	.96 ± .40	45.24 ± 3.54	56.68 ± 2.30	67.18 ± 4.19

Experiments 8 and 9

The objective of experiments 8 and 9 was to det rmine if the addition of the vitamin D_3 derivative 1,25-$(OH)_2D_3$ to a corn-soybean meal diet would enhance the utilization of phytate phosphorus in chickens, with and without exposure to ultraviolet light.

In each experiment, the four different treatments set forth below were each administered to six pens containing ten birds per pen.

1. No ultraviolet light exposure; a basal diet lacking supplemental 1,25-$(OH)_2D_3$.

2. Ultraviolet light exposure; a basal diet lacking supplemental 1,25-$(OH)_2D_3$.

3. No ultraviolet light exposure; a basal diet containing 5 micrograms 1,25-$(OH)_2D_3$ per kilogram of diet.

4. Ultraviolet light exposure; a basal diet containing 5 micrograms 1,25-$(OH)_2D_3$ per kilogram of diet.

All chickens were housed in batteries in which fluorescent lights were on 24 hours each day in the room and in the cages. The fluorescent lights used in the batteries were General Electric, F15T8-CW, with no diffusers, providing 3.4% of the watts in the ultraviolet range (200-400 nm). The lights in half the pens were sleeved with a plastic tube to filter out all ultraviolet light.

In experiment 8 the dietary calcium and phosphorus levels were 0.75% Ca and 0.58% P. In experiment 9 the dietary calcium and phosphorus levels were 0.63% Ca and 0.50% P. All chickens were fed the corn-soybean diet containing the specifications above, and a vitamin mixture supplying 1100 ICU D_3/kg of diet.

The results are shown in Tables 10-13 below.

Analysis of Experiments 8 and 9 Results

The exposure to ultraviolet light had no effect on weight but increased bone ash, reduced tibial dyschondroplasia and calcium rickets in both experiments, reduced phosphorus rickets in experiment 8, and increased calcium, phosphorus, and phytate phosphorus retention in both experiments (Tables 10, 11, 12 and 13).

The addition of $1,25-(OH)_2D_3$ to the diet increased bone ash; decreased the incidence of tibial dyschondroplasia and phosphorus rickets, particularly in the diet containing the low Ca (0.63%) and P (0.50%) in Experiment 8; and significantly increased the amount of phytate phosphorus utilized as measured by phosphorus retention in both experiments. Overall, the addition of $1,25-(OH)_2D_3$ gave maximum phytate phosphorus retention in the presence or absence of ultraviolet light with the exception of one incidence in experiment 8 (16 day retention), in which the combination of ultraviolet light and $1,25-(OH)_2D_3$ promoted significantly greater phytate utilization.

TABLE 10. **Effect of ultraviolet light and dietary supplemental 1,25-$(OH)_2D_3$ on phytate utilization, growth, incidence of tibial dyschondroplasia and Ca rickets, and blood plasma Ca and P levels.**

Experiment 8

Treatments	Received UV light	16-day wt. g	G/F	Bone ash %	Incidence tibial dyschondroplasia	Incidence Ca rickets %	Blood plasma Ca	Blood plasma P
							— mg/100 ml	
Control	Yes	473	.620[ab]	38.0	6[b]	68	10.34	7.10
Control	No	478	.642[a]	37.8	32[a]	53	10.80	6.62
+ 5 μg/kg 1,25-$(OH)_2D_3$	Yes	458	.583[b]	38.2	6[b]	60	11.69	6.07
+ 5 μg/kg 1,25-$(OH)_2D_3$	No	485	.617[ab]	39.0	25[ab]	70	10.56	7.47
Means ± SEM		474 ±	.615 ±	38.2 ±	17 ±	63 ±	10.85 ±	6.81
±		10	.014	.5	8	10	.50	1.04
ANOVA (Probabilities):								
	df							
Treatments	3	.344	.094	.300	.071	.661	.294	.780
UV light	1	.145	.084	.610	.012	.780	.522	.656
1,25-$(OH)_2D_3$	1	.725	.059	.136	.615	.675	.289	.929
UV light * 1,25-$(OH)_2D_3$	1	.312	.725	.283	.660	.265	.140	.368

TABLE 11. Effect of ultraviolet light and dietary supplemental 1,25-$(OH)_2D_3$ on retention of Ca, P, and P, and phytate phosphorus.

<u>Experiment 8</u>

Treatments	Received UV light	Retention		
		Calcium	Phosphorus	Phytate phosphorus
		%	%	%
Basal	Yes	57.5[b]	46.6	63.3[a]
Basal	No	56.3[b]	43.8	48.8[b]
+ 5 μg/kg 1,25-$(OH)_2D_3$	Yes	60.4[ab]	47.2	74.8[a]
+ 5 μg/kg 1,25-$(OH)_2D_3$	No	65.4[a]	47.5	74.9[a]
Means ± SEM		59.9 ± 2.1	46.3 ± 1.8	65.5 ± 4.0
ANOVA (Probabilities):	df			
Treatments	3	.045	.490	.002
UV light	1	.390	.503	.101
1,25-$(OH)_2D_3$	1	.016	.263	.001
UV light * 1,25-$(OH)_2D_3$	1	.168	.415	.094

TABLE 12. **Effect of ultraviolet light and dietary supplemental 1,25-$(OH)_2D_3$ on phytate utilization, growth, incidence of tibial dyschondroplasia and Ca and P rickets, and blood plasma Ca and P levels.**

Experiment 9

Treatments	Received UV light	16-day wt.	G/F	Bone ash	Incidence tibial dyschondroplasia	Incidence calcium rickets	Incidence phosphorus rickets	Blood plasma Ca	Blood plasma P
		g		%	%	%	%	— mg/100 ml —	
Control	Yes	374	.686^{ab}	33.1^{b}	20^{b}	41^{b}	17^{ab}	11.67	2.58^{b}
Control	No	383	.745^{a}	31.3^{c}	47^{a}	66^{a}	34^{a}	11.54	2.13^{b}
5 μg/kg 1,25-$(OH)_2D_3$	Yes	360	.647^{b}	36.1^{a}	2^{c}	11^{c}	7^{b}	10.88	5.08^{a}
5 μg/kg 1,25-$(OH)_2D_3$	No	382	.687^{ab}	36.5^{a}	11^{bc}	25^{bc}	2^{b}	10.89	3.62^{ab}
Means ± SEM		374 ± 14	.691 ± .019	34.2 .3	20 ± 5	36 ± 8	15 ± 7	11.25 ± .42	3.35 ± .58
ANOVA (Probabilities):	df								
Treatments	3	.637	.015	<.001	<.001	.001	.033	.416	.008
UV light	1	.292	.018	.007	.003	.022	.427	.888	.165
1,25-$(OH)_2D_3$	1	.603	.020	<.001	<.001	<.001	.011	.103	.276
UV light * 1,25-$(OH)_2D_3$	1	.603	.619	<.001	.088	.538	.153	.860	.625

TABLE 13. Effect of ultraviolet light and dietary supplemental 1,25-$(OH)_2D_3$ on retention of Ca, P, and phytate phosphorus.

Experiment 9

Treatments	Received UV light	8 Day Retention Calcium	8 Day Retention Phosphorus	8 Day Retention Phytate phosphorus	16 Day Retention Calcium	16 Day Retention Phosphorus	16 Day Retention Phytate phosphorus
		%	%	%	%	%	%
Control	Yes	37.5^{a}	48.0^{b}	64.0^{b}	43.7	56.1^{b}	70.3^{bc}
Control	No	16.1^{b}	40.9^{c}	42.0^{c}	42.0	60.8^{a}	62.8^{c}
5 μg/kg 1,25-$(OH)_2D_3$	Yes	49.6^{a}	54.3^{a}	80.6^{a}	46.1	62.1^{a}	81.8^{a}
5 μg/kg 1,25-$(OH)_2D_3$	No	47.8^{a}	54.3^{a}	79.8^{a}	44.3	63.0^{a}	77.0^{ab}
Means ± SEM		37.8 ± 4.0	49.4 ± 1.8	66.6 ± 3.0	44.0 ± 3.7	60.5 ± 1.0	73.0 ± 3.2
ANOVA (Probabilities):	df						
Treatments	3	<.001	<.001	<.001	.887	<.001	.002
UV light	1	.009	.063	.001	.637	.009	.066
1,25-$(OH)_2D_3$	1	<.001	<.001	<.001	.531	<.001	.001
UV light * 1,25-$(OH)_2D_3$	1	.023	.063	.002	.997	.072	.669

Experiment 10

The objective of experiment 10 was to determine if the addition of the vitamin D_3 derivative 1,25-$(OH)_2D_3$ to a corn-soybean meal diet would enhance the utilization of phytate phosphorus in different broiler crosses, sold by different breeders and having different genetics.

Eighty cockerels were obtained from each of the following breeders: Peterson X Arbor Acres, Peterson X Ross, and Ross X Ross. Each breed was placed in eight pens of ten birds each. The birds in half the pens of each breed were fed the basal corn-soybean meal diet; the remaining birds were fed the basal corn-soybean meal diet supplemented with 5 microgram/kg 1,25-$(OH)_2D_3$/kg diet. All birds were fed a vitamin mixture supplying 1100 ICU D_3/kg of diet. The fluorescent lights were sleeved in all pens to prevent exposure to non-natural ultraviolet light during this experiment.

The results are shown in Tables 14-15 below.

Analysis of Experiment 10 Results

In the criteria measured, a significant breed effect appeared only in the incidence of tibial dyschondroplasia, the Peterson X Arbor Acres cross having a higher incidence (Tables 14 and 15). The addition of 1,25-$(OH)_2D_3$ resulted in significantly increased utilization of phytate phosphorus indicated by increased phytate phosphorus retention in all three of the commercial crosses tested in this study.

TABLE 14. Effect of supplemental dietary 1,25-$(OH)_2D_3$ on phytate phosphorus utilization, growth, and incidence of tibial dyschondroplasia, Ca rickets, and P rickets in common commercial broiler crosses.

Experiment 10

Broiler crosses	Dietary 1,25-$(OH)_2D_3$	16-Day wt.	G/F	Bone ash	Incidence tibial dyschondroplasia	Incidence Ca rickets	Incidence P rickets
	µg	g		%	%	%	%
Peterson X Arbor Acres	0	397	.734	30.1[b]	51[a]	11[ab]	50[b]
Peterson X Arbor Acres	+5	417	.751	35.3[a]	30[b]	10[ab]	13[c]
Peterson X Ross	0	400	.753	29.9[b]	29[b]	12[a]	64[ab]
Peterson X Ross	+5	421	.716	34.7[a]	28[b]	0[b]	13[c]
Ross X Ross	0	381	.745	29.3[b]	29[b]	5[ab]	75[a]
Ross X Ross	+5	390	.723	35.7[a]	25[b]	5[ab]	8[c]
Means ± SEM		401 ± 18	.737 ± .017	32.5 ± .8	32 ± 5	7 ± 4	37 ± 7
ANOVA (Probabilities):	df						
Treatments	5	.574	.654	<.001	.024	.151	<.00
Crosses	2	.318	.885	.666	.033	.258	.35:
1,25-$(OH)_2D_3$	1	.264	.365	<.001	.050	.162	<.00
Crosses * 1,25-$(OH)_2D_3$	2	.930	.351	.177	.141	.147	.13

TABLE 15. **Effect of supplemental dietary 1,25-$(OH)_2D_3$ on phytate phosphorus, Ca, and P retention in common commercial broiler crosses.**

Experiment 10

Broiler crosses	Dietary 1,25-$(OH)_2$D3	Retention: Calcium	Retention: Phosphorus	Retention: Phytate phosphorus
	μg/kg	%	%	%
Peterson X Arbor Acres	0	46.9[b]	50.6[a]	49.6[c]
Peterson X Arbor Acres	+5	47.6[b]	59.3[a]	71.3[ab]
Peterson X Ross	0	43.4[b]	49.2[c]	54.3[c]
Peterson X Ross	+5	50.6[b]	57.3[ab]	67.2[b]
Ross X Ross	0	44.0[b]	52.1[bc]	50.8[c]
Ross X Ross	+5	65.4[a]	60.9[a]	78.9[a]
Means ± SEM		49.6 ± 3.6	54.9 ± 2.0	62.0 ± 3.3
ANOVA (Probabilities):	df			
Treatment	5	.005	.003	<.001
Crosses	2	.082	.310	.352
1,25-$(OH)_2D_3$	1	.004	<.001	<.001
Crosses * 1,25-$(OH)_2D_3$	2	.032	.983	.093

Experiment 11

The objective of experiment 11 was to determine if the addition of large amounts of vitamin D_3 [3300 ICU (82.5 micrograms) or 7700 ICU (192.5 micrograms)] to a basal corn-soybean meal diet already containing 1100 ICU (27.5 micrograms) per kilogram of diet would elicit responses comparable to those obtained from the addition of 10 microgram/kg 1,25-$(OH)_2D_3$/kilogram diet.

The fluorescent lights were sleeved in all pens to prevent exposure to non-natural ultraviolet light during this experiment.

Each of the six different dietary treatments set forth below were provided to four pens containing ten birds per pen.

1. One basal diet lacking supplemental vitamin D_3 or 1,25-$(OH)_2D_3$.

2. One basal diet lacking supplemental 1,25-$(OH)_2D_3$ and containing 3300 ICU vitamin D_3 per kilogram of diet.

3. One basal diet lacking supplemental 1,25-$(OH)_2D_3$ and containing 7700 ICU vitamin D_3 per kilogram of diet.

4. One basal diet containing 10 micrograms/kg 1,25-$(OH)_2D_3$ per kilogram of diet and lacking vitamin D_3.

5. One basal diet containing 10 micrograms/kg 1,25-$(OH)_2D_3$ and 3300 ICU vitamin D_3 per kilogram of diet.

6. One basal diet containing 10 micrograms/kg 1,25-$(OH)_2D_3$ and 7700 ICU vitamin D_3 per kilogram of diet.

All chickens were fed a corn-soybean meal diet containing 1.0% calcium, 0.5% phosphorus, and .25% phytate phosphorus, and a vitamin mixture supplying 1100 ICU D_3/kg of diet.

The results are shown in Table 16 below.

Analysis of Experiment 11 Results

While the addition of either of the two levels of vitamin D_3 or the 1,25-$(OH)_2D_3$ had no effect on 16 day body weights, the highest vitamin D_3 level (7700 ICU/kg) resulted in a significant decrease in the gain/unit of feed consumed. The 1,25-$(OH)_2D_3$ supplementation resulted in decreased incidence and score of tibial dyschondroplasia and dramatic increases in blood plasma phosphorus and bone ash, both indicating significant increases in the utilization of phytate phosphorus by the 1,25-$(OH)_2D_3$ supplemented birds. The high levels of vitamin D_3 resulted in slight but significant increases in dialyzable phosphorus and bone ash.

TABLE 16. Effect of high levels of vitamin D_3 dietary supplementation, with and without supplemental 1,25-$(OH)_2D_3$ on growth, phytate phosphorus utilization, blood plasma Ca and P levels, and incidence of tibial dyschondroplasia.

Experiment 11

Supplementation Vitamin D_3	1,25-$(OH)_2D_3$	16-Day wt.	G/F	Bone ash	Incidence tibial dyschondroplasia	Blood plasma Ca	Blood plasma P
ICU/kg	μg/kg	g		%	%	— mg/100 ml —	
0	0	405	.688^{a}	29.1^{c}	13^{ab}	12.29	2.46^{b}
3300	0	405	.678^{a}	33.0^{b}	22^{a}	11.86	3.61^{b}
7700	0	409	.636^{ab}	34.2^{b}	11^{ab}	11.74	3.66^{b}
0	10	404	.670^{a}	37.5^{a}	3^{b}	11.79	6.19^{a}
3300	10	397	.651^{ab}	37.0^{a}	3^{b}	11.27	6.19^{a}
7000	10	389	.605^{b}	37.1^{a}	5^{b}	10.94	7.29^{a}
Means ± SEM		401 ± 9	.655 ± .018	34.6 ± .5	9 ± .2	11.65 ± .44	4.90 ± .59
ANOVA (Probabilities):	df						
Treatments	5	.619	.042	<.001	.033	.370	<.001
Vitamin D_3	2	.807	.012	<.001	.515	.300	.182
1,25-$(OH)_2D_3$	1	.193	.098	<.001	.003	.097	<.001
Vitamin D_3 * 1,25-$(OH)_2D_3$	2	.529	.931	<.001	.288	.942	.572

Example 2: Treatment of Tibial Dyschondroplasia with Vitamin D_3 Derivatives

The effectiveness of 1,25-dihydroxycholecalciferol (2), 1,25-dihydroxy-26,27-hexadeuterocholecalciferol (3), 1-hydroxycholecalciferol (4), 1,24,25-trihydroxy-cholecalciferol (5), and 1,25-dihydroxy-24-fluorocholecalciferol (6) in reducing the incidence of tibial dyschondroplasia in chickens has been demonstrated using the following experimental procedures.

Newly hatched Peterson X Arbor Acre cockerels obtained from a commercial hatchery were housed as described in Example 1 above. The basal diet used is described above in Table 2. Disulfiram was added to the basal diet of some chickens as indicated in Table 17 at a level of 30 mg/kg of feed. The following levels of vitamin D_3 and its metabolites were added to the diet as indicated in Tables 17 and 18:

Vitamin D_3, 27.5 micrograms per kilogram of feed (1100 ICU/kilogram); and

1,25-dihydroxycholecalciferol (2), 1,25-dihydroxy-26,27-hexadeuterocholecalciferol (3), 1-hydroxy-cholecalciferol (4), 1,24(R),25-trihydroxy-cholecalciferol (5), 1,25-dihydroxy-24(R)-fluoro-cholecalciferol (6), and 25-hydroxycholecalciferol (7) at 10 micrograms per kilogram.

At 16 days of age, pen body weights were obtained for all birds and their feed consumption was recorded. The birds were then sacrificed and examined at random for tibial dyschondroplasia by making a longitudinal cut across the tibia and scoring for incidence and severity of tibial dyschondroplasia as described in Example 1 above. After the tibia was scored for incidence and severity of tibial dyschondroplasia, the left tibia was removed for a bone ash determination on the fat-free dried bone.

Absorption and retention of dietary calcium was measured by orally dosing chicks with ^{47}Ca and then measuring the total body radioactive count, as described in Suso and Edwards, "A Study of Techniques for Measuring ^{65}Zn Absorption and Biological Half-life in the Chicken," *Poult. Sci.* 47:991-999 (1968). Specifically, ten seven day old birds in one pen under the indicated dietary treatments were dosed orally with 0.5 milliliter of water solution containing 0.5 microcuries of ^{47}Ca and 0.1 to 1.0 micrograms of calcium. The total body radioactive content of the individual chickens was determined immediately following dosing, and also on days 7, 9, 12 and 14 following dosing. A plot of percent administered ^{47}Ca which was retained versus number of days yields a line which has a slope corresponding to the half-life of the radioactive calcium in the chicken, and a y-intercept that corresponds to the amount of calcium absorbed by the chicken.

<u>Effect of vitamin D_3 derivatives on Tibial Dyschondroplasia.</u>

The effect in broiler cockerels of vitamin D_3 (<u>1</u>), 25-hydroxycholecalciferol (<u>7</u>), and 1,25-dihydroxy-cholecalciferol (<u>2</u>) on growth, feed efficiency, bone ash, ^{47}Ca retention, and incidence and severity of tibial dyschondroplasia was determined in Studies 1 and 2. The results are shown in Table 17 below. The basal diet contained 1100 ICU (27.5 micrograms) vitamin D_3 per kilogram feed. Disulfiram was added as indicated to the diets of some chickens to increase the incidence of the disease in order to further measure the effectiveness of the compounds.

As shown below in Table 17, 1,25-dihydroxychole-calciferol (<u>2</u>), in the presence or absence of disulfiram, reduced the incidence and severity of tibial dyschondroplasia over the basal diet, and

increased bone ash. 25-(OH)-Cholecalciferol (7), in the presence or absence of disulfiram, also reduced the incidence of tibial dyschondroplasia over the basal diet. Further, a diet with 1,25-dihydroxy-cholecalciferol resulted in significantly more dietary ^{47}Ca absorbed than in a basal diet, while increasing the half-life, or retention, of the calcium administered.

Table 18, shown below, illustrates the results of Studies 3 and 4, in which the effect of vitamin D_3 (cholecalciferol) (1), 1,25-dihydroxycholecalciferol (2), 1,25-dihydroxy-26,27-hexadeuterocholecalciferol (3), 1-hydroxycholecalciferol (4), 1,24,25-trihydroxy-cholecalciferol (5), 1,25-dihydroxy-24-fluorochole-calciferol (6), and 24,25-dihydroxycholecalciferol (8), on the incidence and severity of tibial dyschondroplasia, bone ash and absorption and retention of calcium was measured. The basal diet contained 1100 ICU of vitamin D_3 per kilogram feed.

In Tables 17 and 18, values with different superscript letters are significantly different, $P \leq 0.05$.

Studies 3 and 4 substantiated the results of studies 1 and 2 that administration of 10 microgram/kg feed of 1,25-dihydroxycholecalciferol (2) to the bird per day for the life of the bird substantially reduces the incidence and severity of tibial dyschondroplasia over the basal diet, and increases bone ash.

The addition of 24,25-dihydroxycholecalciferol (8) was not effective in preventing the development of tibial dyschondroplasia.

The chickens that received feed containing 1,24,25-trihydroxycholecalciferol (5), 1-hydroxy-cholecalciferol (4), 1,25-dihydroxy-26,27-hexa-deuterocholecalciferol (3) and 1,25-dihydroxy-24-fluorocholecalciferol (6) had a significant reduction in the incidence of tibial dyschondroplasia.

TABLE 17: Effect of Vitamin D_3; 25-OHD_3 and 1,25-$(OH)_2D_3$ on Severity of Tibial Dyschondroplasia With and Without Disulfiram

Study 1

Treatments	16 Day wt	Gain:Feed	Bone ash	Tibial Dyschondroplasia Incidence	Score	#3/total
Basal	416	.696	40.6	42	2.77	10/30
Basal + D_3	451	.734	40.4	26	2.38	4/27
Basal + 25-OHD_3	395	.667	40.2	17	1.50	1/28
Basal + 1,25-$(OH)_2D_3$	421	.670	42.5	13	1.75	1/29
Disulfiram	433	.750	39.9	70	2.71	16/30
Disulfiram + D_3	432	.708	40.0	40	2.80	9/27
Disulfiram + 25-OHD_3	398	.706	39.7	58	2.54	11/30
Disulfiram + 1,25-$(OH)_2D_3$	426	.701	41.9	23	2.33	4/30
Means$\pm$ SEM	420$\pm$13	.704	.032	40.6$\pm$.2	36$\pm$9	2.11$\pm$.48

TABLE 17 (CONT.)

Study 2

Treatments	16 Day wt	Gain:Feed	Bone ash	Tibial Dyschondroplasia Incidence	Tibial Dyschondroplasia Score	#3/total	^{47}Ca Treatments Intercept	^{47}Ca Treatments Slope
Basal	375	.707	40.5	39	1.94	6/29	72	-1.60
" + D_3	368	.647	40.5	31	2.47	5/29	77	-1.88
" + 25OHD_3	392	.713	40.6	27	2.78	7/30	72	-1.59
" + 1,25 $(OH)_2D_3$	361	.661	42.2	13	2.17	2/30	84	-2.85
Disulfiram	394	.671	40.3	65	2.76	14/28	73	-1.59
" + D_3	374	.666	40.3	51	2.74	12/30	72	-1.97
" + 25OHD_3	395	.728	40.5	49	2.76	11/28	74	-1.67
" + 1,25 $(OH)_2D_3$	377	.684	42.0	30	2.44	6/30	79	-2.11
Means ± SEM	380 ± 12	.685 ± .026	40.8 ± .3	38 ± 9	2.51 ± .31		75 ± 3	-1.91 ± .29

TABLE 18: Effect of Vitamin D3 and Vitamin D3 Derivatives on Severity of Tibial Dyschondroplasia

Study 3

Vit D Compound	16 Day Wt.	Gain/ Feed	Tibial Dyschondroplasia					Ca^{47}	
			Incidence	Score	#3/No.	%N3	%N2+3	Ret.	Slope
none	421	$.711^{a}$	63	1.85	11/60	18	37	70^{bc}	-1.01^{b}
D_3	434	$.709^{a}$	82	1.69	13/59	22	34	75^{abc}	-1.13^{b}
1,25 $(OH)_2D_3$	421	$.66^{b}$	56	1.48	5/58	9	16	78^{ab}	$-.72^{ab}$
24,25 $(OH)_2D_3$	424	$.696^{ab}$	78	2.03	17/59	29	53	67^{c}	$-.77^{ab}$
1,24,25 $(OH)_3D_3$	428	$.688^{ab}$	57	1.58	6/60	10	22	76^{abc}	$-.77^{ab}$
1-OHD$_3$	423	$.661^{b}$	42	1.51	3/60	5	17	75^{abc}	$-.95^{b}$
1,25$(OH)_2$-26-27$[^2H]_6D_3$	422	$.687^{ab}$	42	1.30	0/60	0	8	80^{a}	$.15^{a}$
1,25$(OH)_2$-24R-FD$_3$	413	$.663^{b}$	60	1.36	3/60	5	15	72^{abc}	$-.47^{ab}$
Mean ± SEM	423±7	.685±.013		59±7	1.60±15	12±4.5		74±3	-.71±.31

TABLE 18 (CONT)

Study 4

Vit. D Compound	16 Day wt.	Gain/ feed	Bone ash	Tibial Dyschondroplasia Incidence	Score	%#3	#3/Total
None (basal diet)	356^{cd}	$.681^{ab}$	35.07^{c}	54^{a}	2.30^{ab}	35^{a}	19/55
D_3	383^{a}	$.701^{a}$	35.68^{bc}	44^{ab}	2.75^{a}	35^{a}	20/58
$1,25(OH)_2D_3$	355^{cd}	$.652^{b}$	36.88^{a}	18^{d}	1.58^{b}	8^{b}	4/52
$24,25(OH)_2D_3$	351^{d}	$.663^{b}$	35.53^{bc}	53^{a}	2.78^{a}	46^{a}	25/56
$1,24,25(OH)_2D_3$	376^{ab}	$.681^{ab}$	36.07^{b}	23^{cd}	1.89^{ab}	14^{b}	8/57
$1OHD_3$ 372^{abc}	$.655^{b}$	36.77^{a}	29^{bcd}	2.21^{ab}	15^{b}	8/57	
$1,25(OH)_2$-26-27 $[^2H]_6D_3$ 360^{bcd}	$.646^{b}$	37.45^{a}	16^{d}	1.63^{b}	7^{b}	4/58	
$1,25(OH)_2$-24R-FD_3	360^{bcd}	$.661^{b}$	35.48^{bc}	41^{abc}	2.06^{ab}	16^{b}	9/56
Mean ± SEM	364 ± 6	.667 ± .012	36.12 ± .23	34 ± 6	2.15 ± .31	22 ± 5	

Example 3: Enhancement of Phytase Utilization with Vitamin D_3 Derivatives in Turkeys

The effectiveness of 1,25-dihydroxycholecalciferol (2) in enhancing phytate phosphorus utilization in turkeys has been demonstrated using the experimental procedures described in Example 1, except as described below.

Newly hatched turkey poults obtained from a commercial hatchery were used. Animals were housed in electrically heated Petersime battery brooders with wire-mesh floors located in a room in which the temperature was maintained at 22°C. Sunlight was present during the day from large windows. No attempt was made to limit the natural production of vitamin D_3. Feed and water were available to the turkeys at all times. Fluorescent lights were on 24 hours each day in the room and in the cages. The fluorescent lights used in the batteries were General Electric, F15T8-CW, with no diffusers, providing 3.4% of the watts in the ultraviolet range (200-400 nm). The lights in half the pens were sleeved with a plastic tube to filter out all non-natural ultraviolet light.

The objective of the experiment was to determine if the addition of 10 μg 1,25-$(OH)_2D_3$/kg to a corn-soybean meal diet would enhance the utilization of the phytate phosphorus in turkey poults, with and without the presence of phytase and/or ultraviolet light.

Each of the eight treatments set forth below were administered to three pens of eight tom turkeys per pen.

1. Ultraviolet light exposure; a basal diet lacking supplemental phytase and 1,25-$(OH)_2D_3$.

2. Ultraviolet light exposure; a basal diet containing supplemental 600 units phytase per kilogram of diet and lacking 1,25-$(OH)_2D_3$.

3. Ultraviolet light exposure; a basal diet lacking supplemental phytase and containing 10 micrograms 1,25-$(OH)_2D_3$ per kilogram of diet.

4. Ultraviolet light exposure; a basal diet containing supplemental 600 units phytase and 10 micrograms 1,25-$(OH)_2D_3$ per kilogram of diet.

5. No ultraviolet light exposure; a basal diet lacking supplemental phytase and 1,25-$(OH)_2D_3$.

6. No ultraviolet light exposure; a basal diet containing supplemental 600 units phytase per kilogram of diet and lacking 1,25-$(OH)_2D_3$.

7. No ultraviolet light exposure; a basal diet lacking supplemental phytase and containing 10 micrograms 1,25-$(OH)_2D_3$ per kilogram of diet.

8. No ultraviolet light exposure; a basal diet containing supplemental 600 units phytase and 10 micrograms 1,25-$(OH)_2D_3$ per kilogram of diet.

The basal corn-soybean meal diet used is shown in Table 19. It differed from the broiler diet by having increased protein, represented by the increase in soybean meal, and the addition of certain vitamins and minerals to satisfy the higher dietary requirements of the turkey for these nutrients. All turkeys were fed this corn-soybean diet containing the specifications above and a vitamin mixture supplying 1100 ICU D_3/kg of diet.

The results of this experiment are shown in Table 20 below.

Analysis of Results

All the turkey poults in experiment 12 had phosphorus rickets. Since turkeys do not develop tibial dyschondroplasia at this age, none was detected. The ultraviolet light treatment caused no significant response in any of the criteria measured. The phytase supplementation resulted in a significant increase in 8 day weight, gain/feed, blood plasma

calcium, and bone ash, indicating significant increase in the utilization of phytate phosphorus by poults receiving the phytase. The supplemental 1,25-$(OH)_2D_3$ resulted in a significant increase in gain/feed, blood plasma calcium, and bone ash. These increases were less than those resulting from supplemental phytase. However, supplementation with both resulted in a further increase in gain/feed and bone ash, although these increases were not all significant. Nevertheless, the evidence indicated a synergistic effect between phytase and 1,25-$OH)_2D_3$ in increasing calcium and phosphorus utilization by poults receiving both supplements.

TABLE 19. C mpositi n f th turk y control diet.

Ingredient	Amount g/100 g
Ground yellow corn	41.90
Soybean meal (dehulled)	50.00
Poultry fat (stabilized)	4.00
Iodized sodium chloride	0.35
DL-Methionine	0.20
Vitamin premix[1]	0.25
Trace mineral premix[2]	0.10
Se concentrate (0.02% From sodium selenite)	0.05
Limestone	2.31
L-lysine hydrochloride	0.10
Chromic oxide	0.10

[1]Vitamin premix provided the following (in mg/kg diet, except as noted): all-trans-retinyl acetate, 3.6; cholecalciferol, 27.5 μg (1100 ICU); all rac-α-tocopheryl acetate, 17; riboflavin, 4.4; calcium pantothenate, 12; nicotinic acid, 70; choline chloride, 553; vitamin B-12, 9 μg; vitamin B-6, 5; menadione sodium bisulfite, 1.1; thiamin mononitrate, 2.2; folic acid, 3; biotin, 0.3; ethoxyquin, 125.

[2]Trace mineral premix provided the following (mg/kg diet): MnO_2, 222; ZnO, 150; $FeSO_4 \cdot 7H_2O$, 200; $FeCO_3$, 83; $CuSO_4 \cdot 5H_2O$, 29; and $Ca(IO_3)_2$, 15.

TABLE 20. Effects of dietary supplementation with phytase and/or 1,25-$(OH)_2D_3$ on growth, bone ash, and blood Ca and P levels in turkey poults, in the presence and absence of ultraviolet light.

Treatments						Blood plasma	
Received ultraviolet light	Phytase	1,25-$(OH)_2D_3$	8-Day wt.	G/F	Bone ash	Ca	P
	Units/kg	µg/kg	g		%	mg/100 ml	
Yes	0	0	113^{c}	$.508^{b}$	19.2^{cd}	8.73^{b}	4.38^{b}
Yes	600	0	146^{a}	$.745^{a}$	22.9^{b}	12.19^{a}	2.67^{bc}
Yes	0	10	118^{bc}	$.620^{b}$	20.5^{c}	13.63^{a}	1.52^{c}
Yes	600	10	146^{a}	$.800^{a}$	25.1^{a}	13.59^{a}	2.53^{c}
No	0	0	115^{c}	$.500^{b}$	18.0^{d}	7.94^{b}	6.33^{a}
No	600	0	147^{a}	$.764^{a}$	22.0^{b}	11.21^{a}	2.45^{c}
No	0	10	130^{b}	$.592^{b}$	20.3^{c}	13.25^{a}	1.57^{c}
No	600	10	147^{a}	$.794^{a}$	23.4^{b}	13.42^{a}	2.03^{c}
Means ± SEM			133 ± 4	.665 ± .039	21.4 ± .4	11.75 ± .75	2.94 ± .58
ANOVA (Probabilities):	df						
Treatments	7		<.001	<.001	<.001	<.001	<.001
Ultraviolet light	1		.211	.831	.445	.288	.288
Phytase	1		<.001	<.001	.023	.005	.005
Ultraviolet light * phytase	1		.326	.655	.117	.995	.995
1,25-$(OH)_2D_3$	1		.097	.018	<.001	<.001	<.001
Ultraviolet light * 1,25-$(OH)_2D_3$	1		.414	.681	.201	.873	.573
Phytase * 1,25-$(OH)_2D_3$	1		.087	.291	.001	.007	.007
Ultraviolet light * phytase * 1,25-$(OH)_2D_3$	1		.326	.959	.338	.855	.855

I claim:

1. An animal feed composition comprising:

a. vitamin D;

b. between 1 and 10 micrograms per kilogram feed of a vitamin D_3 derivative selected from the group consisting of 1,25-dihydroxycholecalciferol; 1,25-dihydroxy-26,27-hexadeuterocholecalciferol; 1-hydroxycholecalciferol; 1,24,25-trihydroxycholecalciferol; 1,25-dihydroxy-24-fluorocholecalciferol; and 25-hydroxycholecalciferol; and

c. active phytase.

2. The composition of claim 1 wherein the concentration of vitamin D_3 derivative is between 3 and 6 micrograms per kilogram feed.

3. The composition of claim 1 wherein the animal feed has a calcium content of between 0.5 and 1.3% by weight.

4. The composition of claim 1 administered in combination with a source of non-natural ultraviolet light.

5. The composition of claim 1 wherein the amount of phytase is between 30 and 1200 units per kilogram feed.

6. The composition of claim 1 wherein the animal feed is selected from the group consisting of poultry feed and swine feed.

7. A method for enhancing phytate phosphorus utilization in an animal comprising:

a. administering to the animal an effective amount of a vitamin D_3 derivative selected from the group consisting of 1,25-dihydroxy-cholecalciferol; 1,25-dihydroxy-26,27-hexadeuterocholecalciferol; 1-hydroxycholecalciferol; 1,24,25-trihydroxycholecalciferol; 1,25-dihydroxy-24-fluorocholecalciferol and mixtures thereof; and

b. administering to the animal an effective amount of active phytase to further enhance phytate phosph rus utilization,

in combination with animal feed containing 1.3 % calcium by weight or less.

8. The method of claim 7 wherein the calcium content of the feed is between 0.5 and 1.3% by weight.

9. The method of claim 8 wherein the effective amount of vitamin D_3 derivative is between 1 and 10 micrograms per kilogram feed.

10. The method of claim 9 wherein the animal feed has a phosphorus content of between 0.4 and 0.9% by weight.

11. The method of claim 7 wherein the animal is a poultry animal.

12. The method of claim 7 wherein the animal feed is administered in combination with a non-natural ultraviolet light source.

13. The method of claim 7 wherein the animal feed is administered in combination with a non-natural ultraviolet light source.

14. A method of preparing an animal feed composition comprising the steps of:

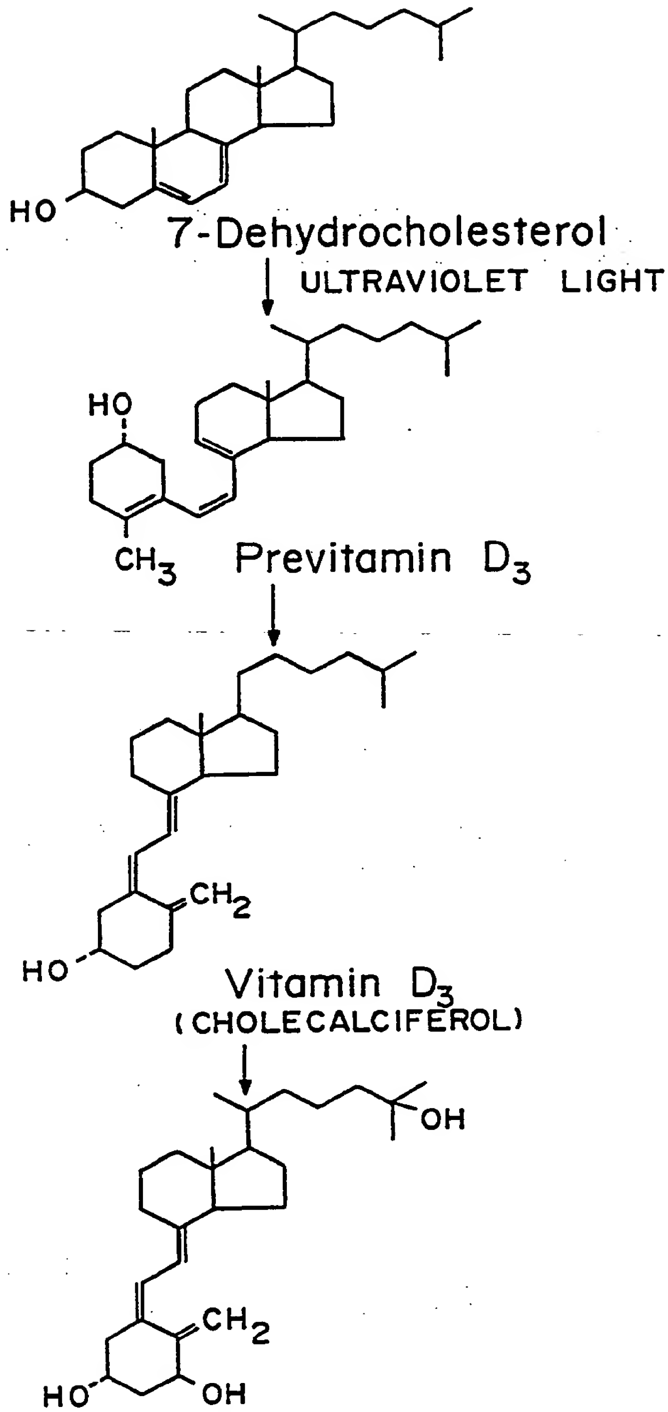
a. adding to the animal feed vitamin D;

b. adding to the animal feed between 1 and 10 micrograms per kilogram feed of a vitamin D_3 derivative selected from the group consisting of 1,25-dihydroxy-cholecalciferol; 1,25-dihydroxy-26,27-hexadeutero-cholecalciferol; 1-hydroxycholecalciferol; 1,24,25-trihydroxychole-calciferol; 1,25-dihydroxy-24-fluorocholecalciferol; and 25-hydroxycholecalciferol; and

c. adding to the animal feed active phytase.

15. The method of claim 14 wherein the animal feed has a calcium concentration of between 0.5 and 1.3% by weight.

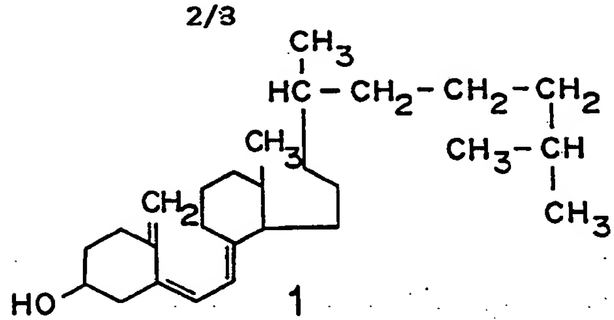
16. The m thod of claim 14 wherein the animal feed has a ph sphorus concentration of between 0.4 and 0.9% by weight.



CALCITRIOL
(1, 25-DIHYDROXYCHOLECALCIFEROL)

FIG. 1
(PRIOR ART)

FIG. 2



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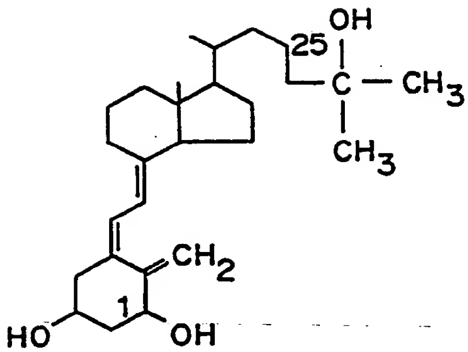

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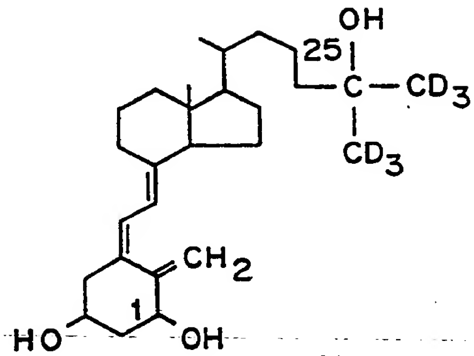

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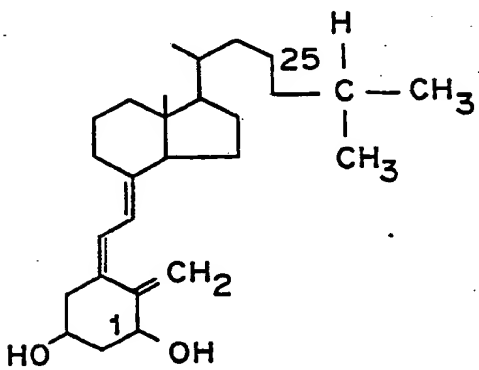

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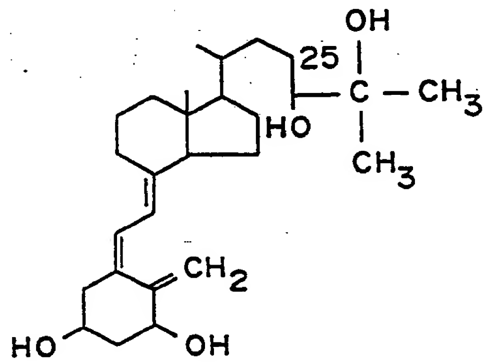

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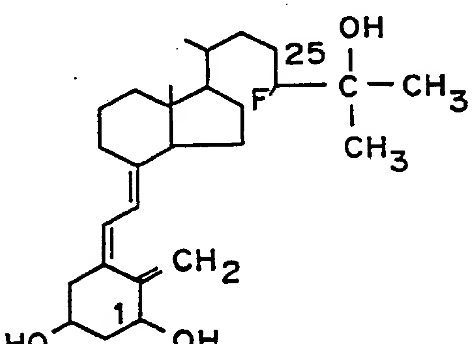

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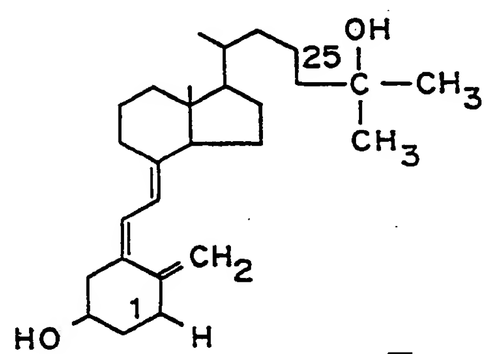

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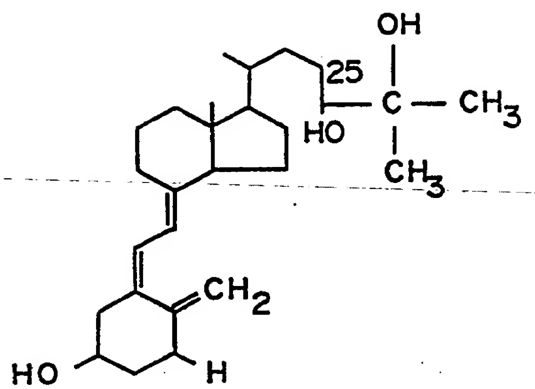

8

FIG. 3

INTERNATIONAL SEARCH REPORT

International application N .
PCT/US93/03012

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 31/59
US CL :424/439, 442; 514/167
According to International Patent Classificati n (IPC) r to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/439, 442; 514/167

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim N .
Y	US, A, 4,929,610 (MEIER ET AL.) 29 MAY 1990 See column 7.	7-11
A	US, A, 5,043,170 (BORENSTEIN ET AL.) 27 AUGUST 1991 See Abstract.	1, 14
A	US, A, 5,082,662 (LAURENT ET AL.) 21 JANUARY 1992; See Abstract.	1, 14

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date		
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 MAY 1993	02 JUL 1993

Name and mailing address of the ISA/US	Authorized officer
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